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SÔBRE "ENTODINA LUNDI" (MÖRCH, 1871) (Mollusca, Pulmonata, Systrophiidae) [1]

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(Com 7 figuras no texto)

Recentemente o meu jovem colega e amigo ANGELO BARBOSA MONTEIRO MACHADO colecionou uma bôa série de conchas de moluscos, algumas provenientes de exemplares recentemente mortos, em pequena gruta de pedra, distante alguns metros da Lapa de Carrancas, no Distrito de S. José da Lapa, município de Vespasiano, Estado de Minas Gerais.

A maior parte destas conchas pertence a *Helicina lundi* Beck *in* Pfeiffer, 1858, encontrando-se entre elas 6 exemplares de *Odontostomus (Bahiensis) costatus* (Pfeiffer, 1848) e cêrca de 50 exemplares de *Entodina lundi* (Mörch, 1871). *Helicina lundi* Beck e *Entodina lundi* (Mörch) foram descritas de material enviado por P. W. LUND e assinaladas pelos autores das espécies como provenientes da "Lapa da Pedra". Não nos foi possível localizar exatamente esta Lapa da Pedra, mas o material em que se baseia o presente trabalho foi encontrado em região e ambiente semelhantes. O município de Vespasiano é próximo a Lagoa Santa, onde LUND viveu quase 50 anos, e está incluido na mesma região de grutas calcáreas desta localidade.

O gênero *Entodina,* creado por ANCEY em 1887, tem como espécie tipo *E. reyei* (Souverbie), descrita do Ecuador, e nele estão incluidas as seguintes espécies, descritas ou referidas no Brasil:

Entodina cheilostropha (d'Orbigny, 1837). Descrita da Bolívia (Santa Cruz de la Sierra) e assinalada no Brasil por MORRETES em 1940. Espécie muito próxima de *E. lundi* (Mörch) pelas estrias transversais numerosas, diferindo por ser maior (6 mm) e por ter maior número de giros (6,5 a 7). A abertura, apesar de semelhante, é muito mais estreita.

Entodina derbyi (Ihering, 1912). Descrita da Bahia, Brasil (Rio Paraguassú), apesar de possuir finas e numerosas estrias transversais, difere das outras espécies do gênero pela forte carena longitudinal externa da concha.

[1] Recebido para publicação a 1 de junho de 1955.

Entodina jekylli Baker, 1913. Descrita de região a 284 Km de Pôrto Velho, na Estrada Madeira-Mamoré, Mato Grosso, tem estrias transversais grossas, sendo a concha achatada, pequena (3,5 mm) e de espiras altas.

Entodina exigua Thiele, 1927. Pequena espécie (2,5 mm), descrita de localidade não especificada, no Brasil, concha quase lisa, com 4,5 giros.

Entodina gionensis Morretes, 1940. Encontrada em Sambaqui, no Município de Iguape, Estado de S. Paulo, tem muita semelhança com a espécie que estudamos, sendo muito provável que se conclua futuramente pela identidade de *E. gionensis* e *E. lundi*. MORRETES em 1940 não tinha conhecimento da publicação da espécie de MÖRCH que escapou a tôdas as referências com exceção da redescrição de PFEIFFER em 1876 como *Helix lundi* e da referência de TRYON (1887) que a coloca na subsecção *Polygyra* juntamente com *E. cheilostropha* d'Orb. A nossa atenção foi despertada pelo nome *lundi* e pelo encontro da espécie ao lado de *Helicina lundi* Beck. Procuramos comparar o nosso material ao material típico de *gionensis* mas não o encontramos no Departamento de Zoologia de S. Paulo, onde atualmente se encontram as coleções do Museu Paulista. A diversidade de região e de ambiente entre o material de *E. lundi* e *gionensis* e a falta da comparação entre os exemplares não nos permitem considerá-las uma única espécie. Transcrevemos a descrição de MÖRCH e a redescrição de PFEIFFER que obtivemos graças a gentileza do Dr. J. Bequaert a quem desejamos agradecer publicamente o grande auxílio que sempre nos tem prestado.

Entodina lundi (Mörch, 1871) comb. n.
(Figs. 1-7)

Helix (Polygyra) Lundii Mörch, 1871 : 38
Helix Lundii Pfeiffer, 1876 : 474
Helix Lundii Tryon, 1887 : 129

Diagnose de MÖRCH:

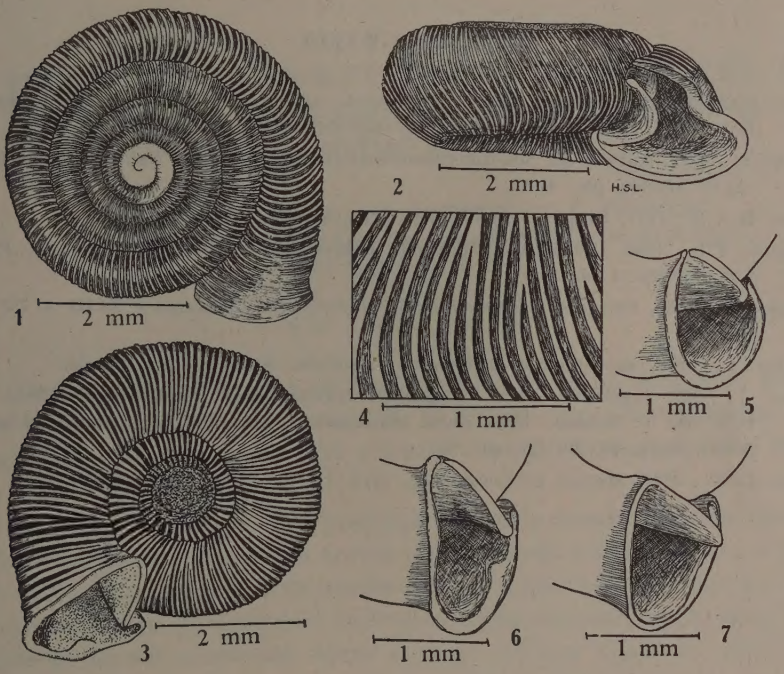
"T. parva, late umbilicata; spira convexiuscula; anfr. 6 angusti, suturis profundis; striae incrementi filiformes, confertae, expressae. Apertura cordiformis, labro crasso albo reflexo, superne dente obtuso munito; callo aperturali auriformi, extus excavato. Diam. maj. 5, min. 4, alt. 1 1/2 mill. Habitat Lappa da Pedro Brasiliae interioris. Lecta a P. W. Lund una cum *Helicina lundii* Beck et *Bul. sulcato* Pfr."

MÖRCH deve referir-se a *B. costatus* Pf.

Redescrição de PFEIFFER:

"T. late et regulariter umbilicata, depressa, subdiscoides, confertissime filoso-costulata, corneo-albida; spira vix convexiuscula; sutura incisa; anfr. 6 angustissimi, convexi, ultimus depresso-rotundatus, non descendens, antice dilatatus, superne ad aperturam subimpressus; umbilicus 1/3 diametri occupans, conicus; apertura diagonalis, subcordiformis; perist. reflexum, marginibus callo arcuato, sublevato junctis, supero in medio dente acuto intus munito. Diam. maj. 5, min. 4, alt. 1 1/2 mill. (Coll. n.º 1465). Helix Lundii Mörch in Malak. Bl. XVIII, p. 38. Habitat Lappa da Pedro Brasiliae interiores (Lund)."

Concha achatada, com 4 a 5,6 mm, composta de 5,5 a 6,75 giros, largamente umbilicada, com 4 a 5,6 de diâmetro menor e 1,5 a 2,4 de altura. Coloração branca ou pérola nos exemplares recentemente mortos. Inteiramente lisa no 1.º ou até o 2.º giro, com indicações de finas costelas transversais do



Entodina lundi (Mörch, 1871) — Fig. 1: Concha, vista dorsal; fig. 2: idem, vista lateral; fig. 3: idem, vista ventral; fig. 4: estrutura das costelas na face ventral; figs. 5 a 7: aberturas das conchas, vista ventral.

fim do 1.º giro até o 3.º. Em alguns exemplares, desde o 2.º giro se estabelecem costelas completas, separadas umas das outras por espaços profundos que variam desde a largura de uma costela até duas ou três vêzes esta largura. Na proximidade da abertura as costelas se afastam ainda mais. Na face inferior da concha as costelas se anastomosam com freqüência, principalmente no último giro. Próximo à abertura o giro se dirige ventralmente e se torna comprimido dorsalmente, projetando-se um calo mais ou menos robusto, para dentro da abertura. Tôda a margem é engrossada nos exemplares adultos e na parede columelar se forma uma lamela que varia um pouco em forma e em desenvolvimento. Esta lamela é achatada, fortemente convexa, fazendo saliência para dentro da abertura, encontra-se geralmente separada da margem engrossada, na parte inferior e está sempre separada por um sulco, na parte superior da abertura.

Material examinado — 6 exemplares adultos e 6 jovens, capturados em gruta sem nome, no distrito de S. José da Lapa, Mun. de Vespasiano, em 1954 (col. I.O.C. 3404); 21 adultos e 20 jovens da mesma gruta, março de 1955 (n.º 3660) colecionados por Angelo Machado.

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Rev. Brasil. Biol., 15 (3) : 247-252
Setembro, 1955 — Rio de Janeiro, D. F.

ESTUDO ELETROFORÉTICO DA REAÇÃO ENTRE A ALBUMINA CRISTALIZADA DE SÔRO BOVINO E O ALDEÍDO FÓRMICO [1]

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(Com 2 figuras no texto)

Dando prosseguimento ao estudo sistemático da reação do aldeído fórmico com as proteínas e ácidos aminados (9, 10, 11) que vem sendo realizado neste laboratório, estudamos desta feita a reação entre a albumina do sôro bovino e o aldeído acima citado.

Dentre os autores que já se preocuparam com o assunto citaremos EDWARDS (1) que procurou reagir o sôro bovino com o aldeído fórmico, CIRCLE & SMITH (2) que estudaram o efeito da reação sôbre o ponto isoelétrico das proteínas, LEULIER & REVOL (3) notando e medindo a acidez crescente com a maior quantidade de formol combinado, MUDD & JOFFE (4) que foram dos primeiros a estudar eletroforèticamente (ou melhor cataforèticamente) a reação em causa, FRAENKEL-CONRAT e colaboradores (5, 6) com seus interessantes trabalhos sôbre a formação de pontes metilênicas intermoleculares, MERIEUX & GORET (7), GUTFREUND & OGSTON (8) e outros.

Para alcançar o nosso objetivo de melhor entender a reação, acompanhamo-la por meio de determinações de aldeído fórmico combinado (12), grupamentos amino livres (13), grupamentos ácidos e básicos totais (14), variações nos espectros de absorção no ultra-violeta e finalmente, determinações das mobilidades eletroforéticas (tanto utilizando eletroforese livre como eletroforese em papel), e é sôbre as últimas determinações que versa o presente trabalho.

Somos gratos ao Dr. A. CURY e ao Snr. P.C.L. PORTELADA, do Laboratório de Microbiologia da Faculdade Nacional de Farmácia, por haverem realizado em seu laboratório a esterilização das soluções estudadas.

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PARTE EXPERIMENTAL

Utilizamos em nossos estudos albumina cristalizada de sôro bovino do Armour Laboratories.

Conforme já descrito nos estudos anteriores com os ácidos aminados (9, 10, 11), preparamos soluções tamponadas a vários pH, contendo algumas 1% de aldeído fórmico e 1% de albumina e outras (testemunhas) contendo sòmente 1% da proteína. As soluções foram incubadas a 37°C; após diferentes intervalos de tempo retiramos as amostras para proceder às análises.

Para evitar contaminação de microorganismos, esterelisamos as soluções por passagem em filtros Seitz EK e as conservamos e manipulamos assèticamente.

Utilizamos nas determinações eletroforéticas o tampão glicina-hidróxido de sódio, com pH 8,4 e fôrça iôntica de 0,2. Na eletroforese em papel, fizemos também algumas determinações qualitativas utilizando um tampão de ácido acético-acetato de sódio, com pH 4,4 e fôrça iôntica de 0,1.

Na necessária diálise que antecede o preparo das amostras para a eletroforese livre, seguimos o método recomendado por LONGSWORTH (15) e utilizamos a última solução tampão da diálise no processo eletroforético.

As eletroforeses livres foram executadas em aparêlho Tiselius, modêlo 38 (Perkin Elmer Co.), em célula de 2 ml, utilizando uma corrente de 19,5 mA, sendo a resistência do tampão de 115 ohms.

Para as eletroforeses em papel, seguimos a técnica descrita por KUNKEL & TISELIUS (16), em aparêlho por nós construido. Nestas experiências utilizamos uma corrente de, aproximadamente 20 mA e 300 volts.

RESULTADOS E DISCUSSÃO

Damos nos quadro e figuras abaixo alguns dos diagramas e mobilidades eletroforéticas das diversas soluções e em tempos variados obtidos na eletroforese livre.

QUADRO I

TEMPO DE REAÇÃO	pH 3		pH 7		ph 11	
	0,0% F	1,0% F	0,0% F	1,0% F	0,0% F	1,0% F
24 horas	5,6	5,4 ; 4,2	5,5	6,4	5,5	7,2
1 semana	5,4 ; 3,7	5,2 ; 3,3	5,5	6,7	5,6	7,7
3 semanas				6,5		8,0
6 semanas	5,4 ; 3,3	5,0 ; 2,7	5,5	6,4	5,4	8,1

As mobilidades são dadas em 10^{-5} cm^2 $volt.^{-1}$ $s.^{-1}$ (eletroforese livre).

Analisando os resultados obtidos, vemos que, enquanto as mobilidades das soluções testemunhas, em pH 7 e 11, mantinham-se constantes, as soluções de idênticos pH que continham aldeído fórmico, tiveram suas mobilidades aumen-

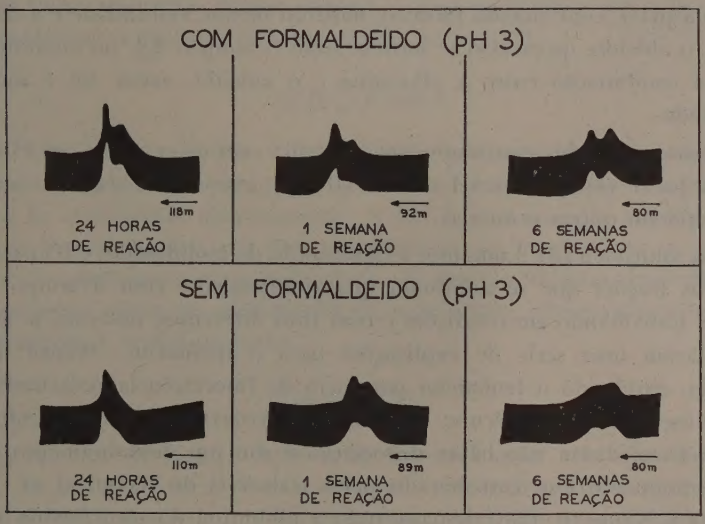

Fig. 1

Fig. 2

tadas. Êstes resultados concordam perfeitamente com as nossas determinações químicas, pois nestes dois pH a reação de MacFayden (12) mostrou ter havido combinação do aldeído, ao mesmo tempo a ninidrina (13) e o Orange G (14)

acusaram diminuição nos grupamentos amino livres; esta diminuição de grupos positivos desloca o ponto isoelétrico da proteína para o lado ácido, aumentando por conseguinte a sua mobilidade. Êste fato foi também amplamente confirmado com o emprêgo de tampão 4,4 na eletroforese em papel, pois a proteína da solução a pH 7, com aldeído fórmico, mostrou menor mobilidade e a da solução 11 com o aldeído, quase não se moveu. Com o tampão 8,4, no entanto, quanto maior a combinação entre a albumina e o aldeído, maior foi a mobilidade encontrada.

Nossos resultados mostram-se concordantes com os expostos por HIRTZ (17), MUDD & JOFFE (4), FISCHER & LAUFFER (18) que executaram trabalho semelhante usando porém outras proteínas.

Nas soluções a pH 3 notamos a diminuição de mobilidades e o aparecimento de novas frações que se acentuam quantitativamente com o tempo[2]. Outros autores, trabalhando em condições e com fitos diferentes, notaram já êstes fatos e desfilaram uma série de explicações para o fenômeno. WEBER (19), por exemplo, estudando o fenômeno por meio de fluorescência polarizada, afirma haver dissociação da molécula; GUTFREUND & STURTEVANT (20), usando método calorimétrico, dizem não haver dissociação e sim um desdobramento da molécula, pensamento êste corroborado pelos trabalhos de BARBU *et al.* (21, 22); PUTNAM & NEURATH (23), desnaturando a albumina do sôro bovino por meio de cloridrato de guanidina, notaram, tal como nós, o aparecimento de outra fração com mobilidade e solubilidade diferentes, outros, tal como FOSTER & YANG (24), afirmam haver exclusivamente maior hidratação e, por conseguinte aumento do volume molecular, fato êste que demonstraram pelo aumento da viscosidade.

A nosso ver, porém, há duas explicações prováveis para os fatos observados a pH 3: 1) desnaturação parcial com desdobramento da molécula devido às repulsões eletrostáticas entre cargas positivas introduzidas pela baixa do pH; êste desdobramento causaria a ruptura de ligações de hidrogênio com exposição de novos resíduos básicos iontisáveis, causando assim a elevação do ponto isoelétrico e consequente diminuição da mobilidade: 2) a desnaturação leva à formação de agregados moleculares, conforme já postulado por BARBU & JOLY (25) e, segundo os recentes trabalhos de HALVER (26) e FRENSDORFF (27) com as albuminas de ovo e de sôro bovino. Entretanto, a menos que nos nossos agregados moleculares haja concomitantemente formação de ligações de hidrogênio entre as carboxilas livres, tal como sugerido por BARBU *et al.*, (22) não vemos como associar êstes agregados com a diminuição da mobilidade.

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[2] Notamos também que ao se fazer a diálise contra água destilada há aparecimento de um precipitado (tanto nas soluções testemunhas como nas que contêm aldeído) que se dissolve na diálise contra o tampão 8,4. Êste fato acentua-se com o passar do tempo de reação, e é mais pronunciado na solução contendo o aldeído fórmico.

Quanto às menores mobilidades da solução com aldeído fórmico, a pH 3, comparadas com as do testemunho, pensamos que sòmente com novos estudos poderão êstes fatos ser esclarecidos.

Os resultados da eletroforese em papel corroboraram "in totum" os da eletroforese livre, revelando porém melhor a heterogeneidade das proteínas em pH 3, conforme também assinalaram BARBU *et al.* (21).

CONCLUSÕES

Pelo visto, a albumina de sôro bovino em pH neutro e básico, sob a ação do aldeído fórmico, tem seu ponto isoelétrico diminuído com o conseqüente aumento da mobilidade eletroforética. A pH 3, com ou sem aldeído, há desnaturação parcial da proteína com aparecimento de frações com maior ponto isoelétrico e, portanto, menor mobilidade. O aldeído fórmico neste pH parece causar pequeno efeito na diminuição da mobilidade, diminuição esta devida, provàvelmente, à acidez do meio.

SUMMARY

The reaction of formaldehyde with bovine plasma albumin in solutions buffered at pH 3, 7 and 11 was investigated electrophoretically after one day, 1, 3 and 6 weeks of reaction. For comparison, blank solutions of albumin were prepared with the same buffers but without formaldehyde and analysed at the same intervals.

At pH 7 and 11 formaldehyde provoked a marked increase in the mobility of the albumin; this effect being stronger at pH 11. This increase in mobility is accompanied by an increase in the amount of formaldehyde bound by the protein.

At pH 3 no increase in mobility nor combination of formaldehyde with the protein was observed. In this pH, the appearance of a new fraction was observed; this fraction as well as the original one showed a slight decrease in mobility. These effects were also detected in a lesser degree, however, in the blank solution of the same pH.

The interpretations of these effects are suggested and discussed.

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FAUNA DO DISTRITO FEDERAL. XXXII.
Um gênero e uma espécie nova de Phaoninae (Diptera, Muscidae) [1]

DALCY DE O. ALBUQUERQUE
Museu Nacional, Rio de Janeiro, D.F.

(Com 6 figuras no texto)

Pachyceramyia gen. n.

Caracteres genéricos — Espaço frontal quase tão largo como longo. Cerdas ocelares robustas, ligeiramente mais fortes que as cerdas pós-verticais internas. Antenas curtas, com ápices arredondados. Arista curta, espêssa, pubescente e pouco maior que o 3.º artículo antenal. Gena e faciália largas. Uma forte cerda protoespiracular dirigida superiormente. Último par posterior de cerdas frontais dirigido para fora. Parafrontália com uma série de cílios fortes. Cerdas dorsocentrais 2+4. Cerda acrostical prescutelar mediocre. Cerdas intralares posteriores, duas. Cerdas esternopleurais 2 : 2 (a ântero-inferior fraca). Escutelo com um par de cerdas basal e um apical, ambos robustos. Asas com nervuras nuas. Calípteros largos, o torácico 1,5 maior que o alar. Tíbia I com uma cerda pré-apical dorsal curta e forte. Tíbia II com duas cerdas posteriores. Tíbia III com duas cerdas ântero-dorsais, uma póstero-dorsal (no limite do I/3 apical) e uma póstero-dorsal, pré-apical. Face dorsal e ântero-dorsal com uma cerda pré-apical.

Espécie tipo — *Pachyceramyia enigmatica* sp. n.

Pachyceramyia enigmatica sp. n.

Fêmea: Cinzenta. Genas largas, arista curta, espêssa e pubescente. Tórax dorsalmente com duas listras castanhas, estreitas, que coincidem com as cerdas dorsocentrais e os cílios acrosticais centrais. Patas castanho escuro com as tíbias amareladas. Asas hialinas.

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[1] Recebido para publicação a 13 de dezembro de 1954.

Comprimento total: 4mm.

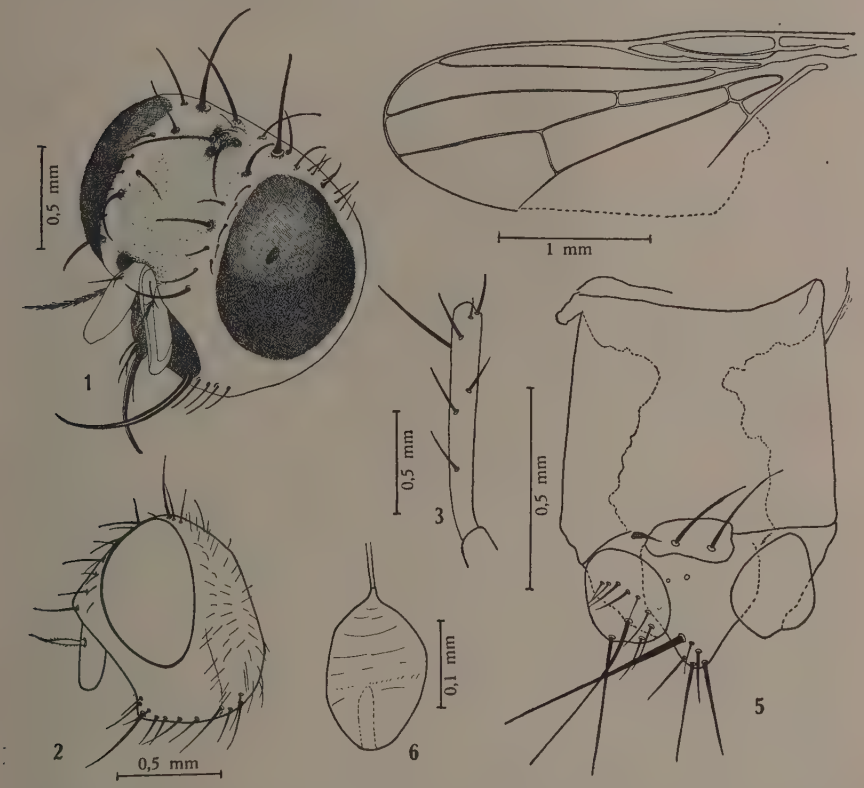
Cabeça — Olhos separados por um espaço mais longo que largo, de bordos paralelos e medindo, à altura do ocelo anterior, cêrca de 2,3 da largura da cabeça. Vita frontal negra, com polinosidade cinzenta, havendo medianamente uma listra prateada fraca, porém distinta. Triângulo ocelar com a mesma coloração da vita frontal. Cerdas frontais seis pares, sendo o último superior, dirigido para fora e os demais convergentes. Dos pares de cerdas frontais, sòmente dois da metade anterior e o superior extrovertido é que são fortes. Órbitas e faciália, prateadas. Triângulo ocelar com um par de cerdas fortes e uma dupla fila de cílios posteriores. Cerdas verticais internas reclinadas, mais fortes que quaisquer dos pares de cerdas frontais. Verticais externas divergentes e tão fortes como o par superior de cerda frontal extrovertido. Cerdas pós-ocelares divergentes, inseridas afastadamente e com cêrca do dôbro das cerdas pós-verticais internas, que são convergentes. Genas prateadas, tão largas quanto o comprimento do 3.º artículo antenal. Parafaciália tão larga como a metade do comprimento do 3.º artículo antenal. Parafrontália com uma série (cêrca de cinco) de cílios fortes, proclinados, iniciados pouco abaixo do último par de cerda frontal superior e terminada pouco antes do nível do último par ântero-inferior. Faciália estreita com um esbôço de carena. Antenas com 1.º artículo amarelo, o 2.º castanho e o 3.º negro. Arista castanha (negra no ápice), pubescente, espêssa e mais curta que a antena. O 3.º artículo antenal mede cêrca de 1,1 o comprimento do segundo. Palpos castanhos e claviformes. Probóscida castanha brilhante.

Tórax — Tórax e pleuras cinzentas. Duas cerdas umerais, uma pós-umeral, uma pré-sutural; 2 + 4 dorsocentrais. Acrosticais em quatro filas de cílios, terminadas por um par de cerdas prescutelares mediocres. Duas cerdas intralares posteriores, uma supralar e duas pós-supralares. Cerdas prototorácicas uma forte e duas pequenas na base, dirigidas superiormente. Cerda protoespiracular uma, dirigida superiormente. Cerdas esternopleurais 2 : 2 (a ântero-inferior fraca, porém distinta de outros pêlos, que existem na esternopleura). Cerdas mesopleurais cinco, sendo a superior dirigida para baixo e uma no ângulo anterior da mesopleura, pouco antes da cerda notopleural anterior. Cerdas notopleurais duas. Escutelo com um par de cerdas basal e um apical, ambos fortes, havendo bem junto a base, um par fraco. Balancim castanho. Calípteros brancos amarelados, o torácico glossiforme e cêrca de 1,5 mais longo que o alar.

Asas hialinas. Nervuras amareladas e núas. Nervulação como na figura.

Patas — Coxas, fêmures e tarsos negros. Ápice dos fêmures e tíbias amarelos. Fêmur I com uma série de cerdas fortes ântero-dorsais e uma série de ântero-ventrais finas. Face dorsal e ântero-dorsal da tíbia com uma cerda pré-apical, sendo esta última, inserida pouco abaixo da primeira. Face ântero-ventral com uma cerda apical. Pretarso com pêlo basal ventral mediocre. Unhas grandes com a metade basal castanha e a apical negra. Pulvilos grandes e branco-amarelados. Fêmur II com duas cerdas posteriores, na metade apical. Face dorsal

e ântero-dorsal com uma cerda pré-apical, inseridas ao mesmo nível. Face anterior, póstero-ventral, póstero-dorsal e ventral com uma cerda apical, forte. Unhas e pulvilos como no par anterior. Fêmur III com uma série de cerdas ântero-ventrais, pouco diferenciadas dos pêlos de fundo, que termina por duas cerdas maiores. Face ântero-dorsal com uma série de cerdas. Face póstero-dorsal com uma cerda pré-apical. Face ventral com cêrca de três cerdas longas e finas na base. Tíbia com uma cerda póstero-dorsal, no limite do 1/3 superior, uma ântero-ventral submediana e duas ântero-dorsais, na metade superior. Face



Pachyceramyia enigmatica sp.n., fêmea — Fig. 1: Cabeça; fig. 2: cabeça, vista de 3/4; fig. 3: tíbia III, vista anterior; fig. 4: asa; fig. 5: terminália, vista dorsal; fig. 6: espermateca.

dorsal e ântero-dorsal com uma cerda pré-apical forte, esta última inserida pouco abaixo da primeira. Face anterior, ântero-ventral e ventral com uma cerda apical, sendo a anterior mais fraca. Unhas e pulvilos como nos pares anteriores.

Abdômen chatô, cinzento, com uma estreita listra castanha mediana, que percorre todos os segmentos. Terceiro segmento com uma série de cerdas marginais apicais e 4.º com discais.

Material examinado: *Holótipo fêmea* n.º 4716, Manguinhos—Praia—(Distrito Federal) Brasil, 25/XII/936, S. Lopes col.

Pachyceramyia gen.n., apresenta caracteres de várias subfamílias de *Muscidae*, entretanto é com *Phaoninae* sua maior afinidade.

Um único par de cerdas frontal dirigido para fora, a ciliação da parafrontália, a largura da parafaciália e da gena, situa distintamente *Pachyceramyia* gen.n. entre os gêneros de *Phaoninae*.

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UEBER DIE TERGITALEN DRUESENFELDER VON "SYNTERMES DIRUS" (Isoptera) [1]

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(Mit 6 Figuren)

Nach dem Schwaermflug der Termiten werfen Maennchen und Weibchen nach dem Erreichen des Erdbodens willkuerlich durch eine heftige, mehrmals wiederholte Schuettelbewegung beide Fluegelpaare ab. Unmittelbar nach dieser Autotomie beginnt das Weibchen seinen Marsch, um einen guenstigen Nistplatz zu finden. Dabei laeuft es mit einer beachtlichen Geschwindigkeit, relativ unempfindlich gegen aeussere Einfluesse, und aendert immer wieder, nicht nur vor Hindernissen, sondern aus freien Stuecken, oft kurze Haken schlagend, seine Laufrichtung.

Waehrend des Fluges kann ein Sich-Suchen der Geschlechter nicht beobachtet werden, ebenfalls reagieren beide Teile nicht aufeinander, bevor sie den Schwaermflug hinter sich haben: Maennchen und Weibchen haben beim Verlassen des Nestes — dieses bezieht sich nur auf die von mir in Rio de Janeiro hacufig beim Schwaermen beobachtete Art *Syntermes dirus*, die grosse Erdbauten aufwirft — nur den einen Trieb, moeglichst schnell com Eingang des Nestes laufend sich zu entfernen, um genuegend Raum zum Oeffnen der doppelt koerperlangen Fluegel zu haben. Waehrend dieser Zeit ist keine andere Instinktaeusserung der Tiere, die sich auf das Suchen des anderen Geschlechts beziehen koennte, zu bemerken. Dieses indifferente Verhalten aendert sich jedoch schlagartig mit dem Augenblick des Landens nach dem Flug. Beide Geschlechter versuchen so schnell wie moeglich, ihre Fluegel abzuwerfen, was im allgemeinen nach einigen kraeftigen Kontraktionen der gesamten Koerpermuskulatur, verbunden mit ruckartig einsetzenden Schuettelbewegungen des Thorax, schnell gelingt, beim Weibchen schneller als beim Maennchen, das haeufig die Fluegel laengere Zeit nach der Landung noch besitzt.

Treffen sich nach dem Flug ein Weibchen und ein noch befluegeltes oder schon ungefluegeltes Maennchen, so macht letzteres alle Anstrengungen, das Weibchen zu erreichen und zu begleiten, wobei dieses jedoch garkeine Notiz

[1] Eingegangen am 6 Juni 1955.

von den Bemuehungen nimmt, sondern mit der gleichen Geschwindigkeit weiterlaeuft und Haken schlaegt. Das Maennchen schiebt sich im Laufen mit seinem Kopf unmittelbar an das Abdomenende des Weibchens und folgt diesem mit ebensolcher Geschwindigkeit und in allen Richtungsaenderungen. Die Reaktionsgeschwindigkeit des Maennchens ist so gross, dass auch bei abrupter Aenderung der Richtung durch das Weibchen sich der Abstand zwischen beiden Tieren nicht aendert. Es erweckt den Eindruck, als ob das Maennchen mit seinen Mandibeln das Weibchen am Hinterende festhalte. Trotz zahlreicher Beobachtungen konnte in keinem Falle ein solcher Vorgang nachgewiesen werden. Trennt man beide Tiere durch ein zwischen ihnen eingeschobenes Blatt oder auch ein duennes Deckglas, um eventuelle optische Reize nicht auszuschalten, so verliert das Maennchen sofort den Kontakt, ist einen Augenblick unorientiert, beginnt dann aber gleich sehr aufgeregt umehr zu suchen; das Weibchen laeuft jedoch ungestoert in der alten Form weiter.

TOPOGRAPHIE DER DRUESEN

Es war nun interessant zu wissen, worauf dieser enge Anschluss des Maennchens an das Weibchen beruht. Optische Reize wurden, wie oben zitiert, ausgeschlossen. Weiterhin ist an taktile Reize, wie auch an chemische, hervorgerufen durch einen vom Weibchen ausgesandten Atraktivstoff, zu denken. Die taktilen Reize koennen a priori ausgeschlossen werden, da — wie oben erwaehnt — vor dem Flug das Weibchen nicht anlockend auf das Maennchen wirkt und da letzteres sich nie einem anderen, ungefluegelten Maennchen anschliesst, das sich aeusserlich bezueglich der Form des Abdomenendes nur wenig vom Weibchen unterscheidet. Ausserdem ergab eine Pruefung der Antennen beider Geschlechter, dass die Anzahl und Verteilung der taktilen Sinnesrezeptoren bei beiden die gleichen sind. Es bleibt damit nur eine chemische Einwirkung auf das Maennchen zu untersuchen.

Es ist interessant zu bemerken, dass mit dem Abwurf der Fluegel beim Weibchen bedeutende Veraenderungen vorgehen: Einmal aendert sich der Bewegungsinstinkt vom planlosen Schwaermflug zum orientierten Suchmarsch fuer eine Nistgelegenheit, andererseits muss es in demselben Augenblick einen Duftstoff aussenden, der eine grosse Atraktivkraft auf das Maennchen ausuebt. So ergab sich die Notwendigkeit, die den Duftstoff sezernierende Druese zu finden, und durch Ausfallversuche den Beweis ihrer Funktion zu erbringen.

Zu diesem Zweck wurden die letzten Abdominalsegmente der Weibchen mit einem Zelluloselack (gewoehnliches Zellophan in Azeton geloest) ueberzogen, der von den Tieren ohne Schaedigung vertragen wird. Dabei stellte sich heraus, dass das Maennchen einem Weibchen gegenueber voellig indifferent bleibt, wenn bei diesem das Hinterleibsende wenigstens vom 8. Segment an vollstaendig ueberzogen ist. Wenn jedoch das ganze Abdomen bedeckt ist mit Ausnahme der letzten Segmente, tritt die Reaktion normal und in voller Staerke ein. Nach

diesen Orientierungsbeobachtungen, die im Park des Instituts Oswaldo Cruz angestellt wurden, wurde material von *Syntermes dirus* auf einer Reise nach Mato Grosso (Salobra) fixiert, um die Druesen endgueltig zu lokalisieren und zu beschreiben.

Aus verschiedenen Arbeiten ueber die weiblichen Atraktivdruesen von Lepidopteren (FREILING, GOETZ, BARTH u.a.) wissen wir, dass sie generell von der Hypodermis der Intersegmentalmembranen der letzten Hinterleibssegmente gebildet werden. Hier bei *Syntermes* (und bei einigen weiteren, nicht bestimmten Arten von Erd- und Baumtermiten) finden sich in den Intersegmentalmembranen des 1. bis 7. Abdominalsegments Trichome mit sehr kleinen Basaldruesen, die als Atraktivdruesen fuer die Maennchen nicht in Frage kommen, da sie einmal bei dem zweiten Orientierungsversuch mit Zelluloselack ausgeschlossen wurden und da sie andererseits in diesem Alter des Weibchens noch klein und unentwickelt sind und histologisch das Bild einer Druesenzelle in absolutem Ruhestadium bieten. Vom 8. Segment an fehlen diese Trichomkomplexe voellig, dafuer liegt auf den Tergiten des 9. und 10. Abdominalsegments je ein geschlossenes Druesenfeld, das von den Tergiten nur soviel Platz beansprucht, wie von dem Tergit des vorhergehenden Segments in der Ruhelage bedeckt wird (Fig. 1). Die Hinterraender der Tergite sind jedoch in keiner Weise veraendert und es findet sich kein Verschlussmechanismus zum Schutz der Druesenfelder. Seitlich reicht die druesige Hypodermis in beiden Segmenten bis an die Pleuralregion heran (Fig. 2 und 3), auch hier nur soweit, wie sie unter dem Schutz des vorhergehenden Segments liegt. Die Fig. 2 gibt einen Schnitt durch den hinteren Teil der Druese des 9. Segments wieder; es erscheinen bereits die vorderen seitlichen Teile der Druese des 10. Segments. In der Fig. 3, durch die Mitte der Druese in X a, ist das Tergit IX a bereits frei von Druesenzellen. Da in diesem Teil des Koerpers, von IX a bis zum Ende, keine weitere Druesen gefunden werden, muessen wir die oben lokalisierten Felder als die Atraktivdruesen fuer die Maennchen ansehen. Es ergibt sich damit, dass diese Druesen bei den Termiten nicht mit denen der Lepidopteren topographisch homologisiert werden koennen, obwohl sie physiologisch und in Bezug auf die Grundgewebeart homologe Organe sind.

MIKROANATOMIE DER DRUESEN

Bedeutender ist jedoch der mikroanatomische Unterschied der Druesen zu denen der Lepidopteren. Waehrend bei diesen die druesigen Hypodermiszellen ihr Sekret direkt, ohne Einschaltung eines Ausleitungsapparates, durch die Cuticula auf osmotischem Wege (oder vielleicht auch durch die Pseudoporen) ausscheiden, wird bei *Syntermes* ein kompliziertes Kanalsystem gebildet, das die Cuticula durchdringt, um das Sekret auf die Koerperoberflaeche zu leiten. Ein Schema eines Teils der druesigen Hypodermis ist in der Fig. 4 dargestellt. Es finden sich zwei Zellarten: Druesenzellen (DZ) und Stuetzzellen (SZ).

Die Druesenzellen haben eifoermige Gestallt und stossen mit ihren apikalen Enden nur eben noch an die Cuticula, oft scheint ein Zusammenhang mit dieser ueberhaupt zu fehlen. Ihr basaler Teil legt sich breit an die Basalmembran an

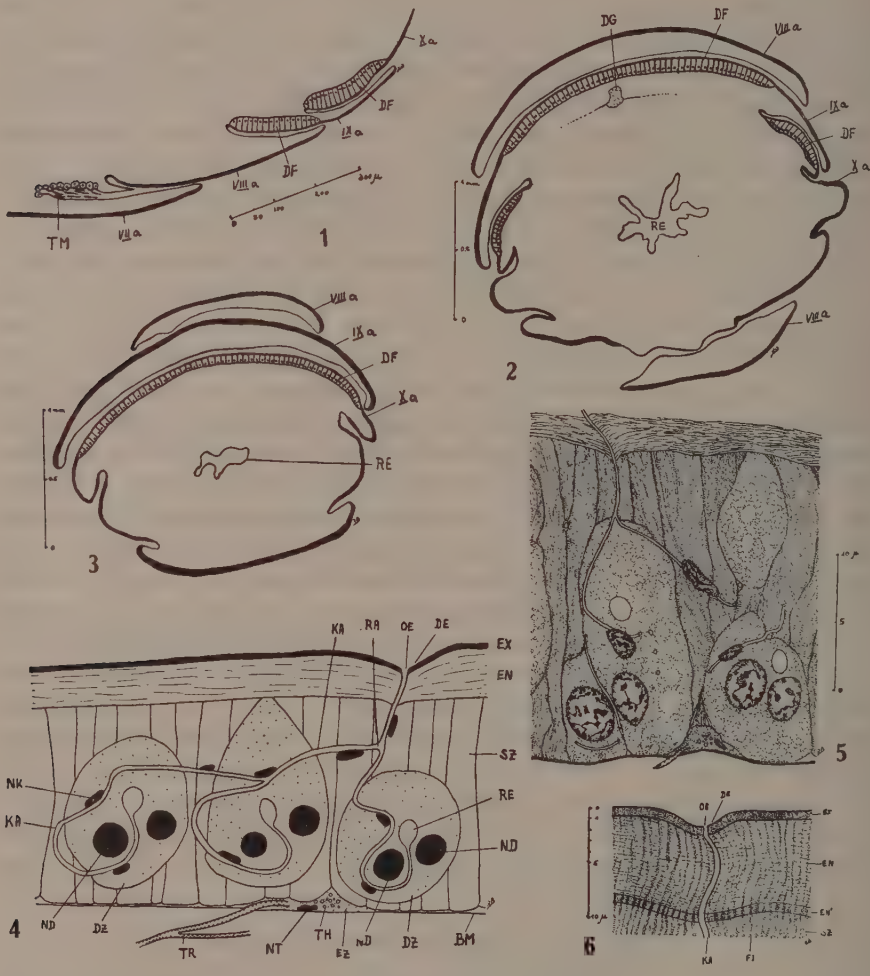

Fig. 1 — Schema der Lage der Druesenfelder. Frontalschnitt, linke Koerperseite. Fig. 2 — Querschnitt durch die hintere Haelfte des Druesenfeldes des 9. Abdominalsegments mit den vorderen seitlichen Teilen des Druesenfeldes des 10. Segments. Fig. 3 — Querschnitt durch die hintere Haelfte des Druesenfeldes des 10. Abdominalsegments. Fig. 4 — Schema der Druesenhypodermis. Kerne schwarz, Druesenzellen punktiert, Stuetzzellen weiss. Fig. 5 — Schnitt durch die Druesenhypodermis. Die Indikationen sind fortgelassen, zur Orientierung siehe Fig. 4. Die Cuticula ist nur angedeutet. Fig. 6 — Cuticula der Druesenfelder mit einer Kanalmuendung. (BM = Basalmembran; DE = Einsenkung der Cuticula; DF = Druesenfeld; DG = Dorsalgefaess; DZ = Druesenzelle; EN = Endocuticula; EN' = innere Endocuticula; EX = Exocuticula; EZ = Tracheenendzelle; FI = Filamente; KA = Kanal; ND = Druesenzellkern; NK = Kanalzellkern; NT = Tracheenendzellkern; OE = Ooeffnung; RA = Verzweigung: RE = Reservatorium; SZ = Stuetzzelle; TH = Tracheole; TM = Trichomorgan; TR = Trachee; VIIa — Xa = 7. — 10. Abdominalsegment).

(in Fig. 4 nicht dargestellt, in Fig. 5 sichtbar). Sie stossen nie oder nur selten an den Seiten aneinander, sondern jede wird von einer Anzahl von Stuetzzellen umgeben. Diese sind lange, zylindrische Zellen, deren Breite oben wie unten gleich ist, nur in der Mitte werden sie, je nach Form der Druesenzellen, eingeschnuert oder aus ihrer senkrechten Stellung zur Seite gedraengt. Die Cuticula besitzt ueber den Druesenfeldern keine Anhaenge oder hervorstechende Strukturen. Beim Aufblick erkennt man eine unregelmaessige Punktierung, die den apikalen Enden deutlicher, senkrechter Strukturen der Endocuticula entsprechen. In gewissen Abstaenden jedoch ist die Cuticula wannenfoermig eingesenkt (Fig. 4 und 6, DE). Am Grund dieser Vertiefung muendet ein Kanal von etwa 0,2 bis 0,3 μ Durchmesser. Dieser durchdringt die Cuticula schraeg oder in schwach gebogenen Verlauf und dringt zwischen den Stuetzzellen hindurch in die Hypodermis ein. Bald schon bildet er zwei Aeste (Fig. 4, RA, auch in Fig. 5 sichtbar), von denen einer zur naechsten Druesenzelle geht, immer zwischen den Stuetzzellen hindurch laufend, ohne in dieselben einzudringen. Der andere Ast bleibt von der Verzweigungsstelle an mehr oder weniger horizontal, gibt noch ein oder zwei Seitenaeste zu anderen Druesenzellen ab und tritt mit seinem Ende an eine ebensolche heran. Nach kurzem oder laengerem Verlauf zwischen den Zellen dringen die Aeste in die Druesenzellen ein. Die Basalmembran ist relativ kraeftig und wird oft von Lymphozyten sekundaer verstaerkt und von Tracheen (Fig. 4, TR) durchbrochen, deren Endzellen (EZ) sich zwischen Druesen- und Stuetzzellen einerseits und der Basalmembran andererseits befinden; in ihnen erkennt man die Querschnitte der feinen Tracheolen (TH).

HISTOLOGIE DER DRUESEN

Die Zellen der gesamten Druesenhypodermis, Druesenzellen wie Stuetzzellen sind jede fuer sich scharf begrenzt. Die Druesenzellen besitzen immer zwei kreisrunde oder nur sehr schwach ovale Kerne mit sehr lockerem Chromatin, das nur zum geringen Teil wandstaendig ist; seine groesseren Kongregate liegen, durch ein kraeftiges Geruest gehalten, im Hohlraum, der reichlich Kernfluessigkeit enthaelt (Fig. 5). Die Doppelkernigkeit von Zellen bei Insekten ist kein seltener Fall; wir treffen solche Zellen in besonders stark aktiven Geweben wie z.B. im Eifollikelepithel bei Homopteren oder in den Speicheldruesen bei Triatominen, Organe mit einem ueberaus regen Stoffaustausch oder kraeftiger, oft zeitlich gebundener Aktivitaetsspitze. Eine solche muessen wir auch fuer die tergitalen Druesen von *Syntermes* annehmen, da die Druese als Atraktivdruese nur kurze Zeit mit voller Produktion arbeitet. Spaeter, in der Hochzeitszelle des neuen Nestes, ist keine Aktivitaetsspitze mehr noetig; ein Fehlen derselben waehrend des Suchmarsches wuerde ein Sich-Finden der Geschlechter in Frage stellen und damit den Bestand der Art gefaehrden.

Das Protoplasma der Druesenzelle ist sehr stark aufgelockert, wabenfoermig oder mit kleineren und groesseren Vakuolen angefuellt, so dass die eigentliche Protoplasmamasse relativ gering ist. Der Kanal oder einer seiner Zweige dringt an irgendeiner Stelle in den Zellkoerper ein, wendet sich zur Basis, laeuft unterhalb eines Kernes zur Mitte und wendet sich zum Zellzentrum. Hierbei passiert er zwischen den beiden Kernen hindurch und endet mit einer blasigen, ovalen Auftreibung (Fig. 4 RE). Auf seinem ganzen Verlauf wird der Kanal von kleinen Kernen begleitet, die sich eng an ihn anlegen und ein dicht gelagertes Chromatin, aber wenig Kernfluessigkeit enthalten (Fig. 4 NK). Die Kanalwand ist gegen das umgebende Protoplasma scharf abgegrentz; ihre Substanz duerfte der Cuticula entsprechen, doch war der Nachweis wegen ihrer Feinheit (vermutlich weniger als 0,05 μ) nicht einwandfrei moeglich. Da aber an Risstellen der Kanal zumeist erhalten bleibt, wird seine cuticulare Natur wahrscheinlich. Hierfuer spricht ebenfalls die vermutliche Entstehung des Kanalsystems als Invagination der Hypodermis. Ob die Endblase (Fig. 4, RE) aus derselben Substanz besteht oder ob sie eine zelleigene Struktur plasmatischer Herkunft ist, bleibt ungewiss. Das Protoplasma der Druesenzelle an der Endblase ist in keiner Weise differenziert, wie wir es sonst oft bei den maennlichen Duftdruesen der Lepidopteren oder anderen Hautdruesen der Insekten im allgemein finden. Ein Protoplasma des Kanalsystems in der Druesenzelle wie auch im gesamten Verlauf des Kanals war nicht einwandfrei zu erkennen.

Die Stuetzzellen (Fig. 4 SZ) besitzen ein sehr dichtes Protoplasma, das in kraeftigen parallelen Laengsfilamenten angeordnet ist. Ihre Basis setzt sich fest der Basalmembran auf und laesst keine oder nur sehr kleine Interzellularraeume erkennen (Fig. 5, links unten). Da im Schnitt der Fig. 5 die Cuticula nicht einwandfrei geschnitten war, wurde ein Ausschnitt aus einem anderen Schnitt in Fig. 6 vergroessert dargestellt. Die Filamente (FI) der Stuetzzellen setzen sich direkt in Vertikalstrukturen der ganzen Endocuticula fort. Dabei erleiden diese Strukturen in den unteren Schichten eine Verstaerkung, so dass von der Endocuticula eine basale Schicht scharf abgegrenzt ist (EN'). In dieser ist ebenfalls die chemische Komposition der horizontalen Schichten anders als die der uebrigen Endocuticula, was durch unterschiedliche Affinitaet zu Farbstoffen zum Ausdruck kommt: Mit Eisenhaematoxylin faerbt sich diese Schicht mit fast derselben Intensitaet wie die Exocuticula. Aus diesem Befunde laesst sich schliessen, dass die Stuetzzellen fest mit der Cuticula verbunden sind. Die Kerne dieser Zellen, immer in Einzahl, sind wesentlich kleiner als die Druesenzellkerne, besitzen dichteres Chromatin und sind lang oval mit unregelmaessigem Umriss, oft nierenfoermig; ihre Laensgsachse laeuft der der Zelle parallel. Ihre Position in der Zelle ist variabel. Die Stuetzzellen sind nicht druesiger Natur, wie aus dem Aufbau des Protoplasmas und aus dem Mangel eines Ausfuehrapparats hervorgeht.

Verschiedene Funktionsstadien der Druesenzellen wurden nicht gefunden; sie sind auch andererseits nicht zu erwarten, da die Druesenzellen nur eine

relativ kurze Zeit ihre volle Taetigkeit entwickeln muessen, eben nur zur Zeit des Suchmarsches des Weibchens. Zu entscheiden, ob diese Druese nun im spaeteren Leben des Weibchens als "Koenigin" des neuen Nestes dafuer verantwortlich zu machen ist, dass das Maennchen Zeit seines Lebens an ihrer Seite ausharrt und die Kopulation wiederholt ausgefuehrt wird, liegt ausserhalb des Ziels dieser Arbeit.

ZUSAMMENFASSUNG

Es wird ein druesiges Organ in der Hypodermis der Tergite des 9. und 10. Abdominalsegments des Weibchens von *Syntermes dirus* beschrieben. Dieses Organ fehlt dem Maennchen. Es handelt sich um zwei Felden von Druesenzellen, die von Stuetzzellen eingeschlossen werden. Gruppen von drei oder vier Druesenzellen stehen durch einen verzweigten Kanal aus Cuticularsubstanz mit der Oberflaeche in Verbindung. Der Kanal dringt bis in das Zentrum der Zelle ein und endet hier mit einer Endblase; er ist von kleinen Kanalzellen, die mit urspruenglichen Hypodermiszellen zu homologisieren sind, begleitet. Durch Verschliessen der druesigen Segmente mit Zelluloselack wird es wahrscheinlich gemacht, dass das Sekret das Maennchen anlockt und dieses veranlasst, dem Weibchen auf der Suche nach einem Nistplatz zu folgen.

RESUMO

É descrito um órgão glandular na hipoderme dos tergitos dos 9.º e 10.º segmentos abdominais da fêmea de *Syntermes dirus*. Êste órgão é ausente no macho. Trata-se de duas áreas de células glandulares incluidas em células de apôio. Grupos de 3 ou 4 células glandulares comunicam-se com a superfície por intermédio de um canal ramificado, composto de substâncias cuticulares. O canal penetra até o centro da célula terminando, aí, por uma vesícula; êle é acompanhado por pequenas células maternas que correspondem às células hipodérmicas primitivas. Cobrindo os segmentos glandulares com soluto de celulose prova-se que a secreção atrai o macho, pois, sem o revestimento do soluto, êle acompanha a fêmea na procura de um lugar para um novo ninho.

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Rev. Brasil. Biol., 15 (3) : 265-270
Setembro, 1955 — Rio de Janeiro, D. F.

ESTUDO ELETROFORÉTICO DA REAÇÃO DO FORMALDEÍDO COM AS PROTEÍNAS DO SÔRO [1]

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(Com 3 figuras no texto)

A reação do formaldeído com as proteínas tem sido objeto de numerosos estudos, entre os quais há alguns que se referem à reação com as proteínas do sôro humano e do sôro bovino. Os trabalhos de STRAUB, VIOLLIER & MÜLLER (1) mostram que o formaldeído provoca, mesmo na temperatura ambiente, alteração do diagrama eletroforético, no qual as globulinas tendem a se confundir com as albuminas. GUTFREUND (2) aqueceu sôro bovino com formaldeído e verificou aumento de heterogeneidade eletroforética das frações e modificação das respectivas mobilidades. HIRTZ (3), em seu trabalho, conclui ser provável que o aldeído fórmico provoque a condensação das diversas moléculas proteicas com aparecimento de novas frações com maior mobilidade que a albumina do sôro original.

Em seqüência ao estudo da reação da albumina cristalizada de plasma bovino com o formaldeído, realizado neste laboratório (4), apresentamos neste artigo uma análise do comportamento eletroforético do sôro humano em presença do formaldeído a 37°C, em função do pH e da concentração de aldeído fórmico.

PARTE EXPERIMENTAL

Utilizando sôro humano normal, cedido gentilmente pelo Banco de Sangue da Prefeitura do Distrito Federal, preparamos soluções contendo cada uma volumes iguais de sôro, solução tampão e solução de formaldeído ou então, no lugar desta última, água destilada. Os tampões [2] usados foram de pH 3, 7 e 11 e as concentrações das soluções de formaldeído foram respectivamente

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[2] O tampão utilizado é constituído pela mistura de ácidos bórico, acético e fosfórico de concentrações 0,12M e hidróxido de sódio 0,2M.

3% e 0,9%. Dêste modo, foram preparadas para cada pH três diferentes soluções contendo respectivamente 1%, 0,3% e 0% de formaldeído, constituindo esta última a solução testemunho[3].

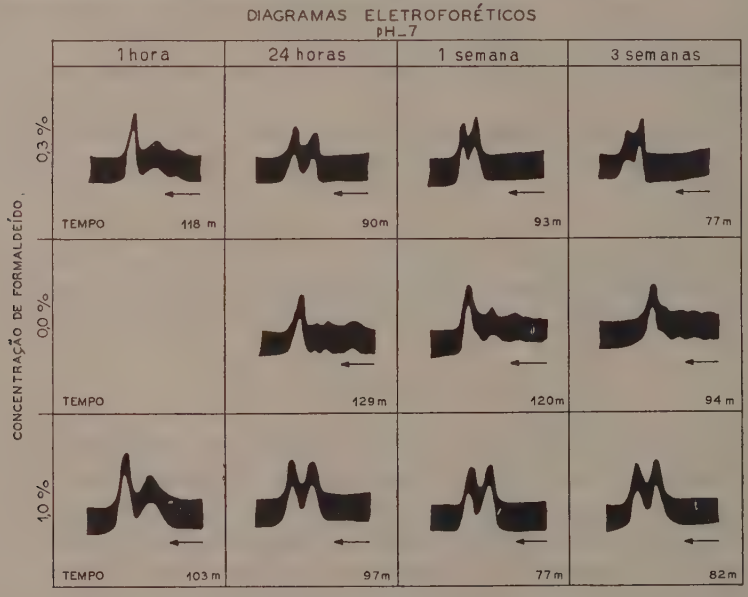
Após a adição do formaldeído, corrigimos o pH de cada mistura, juntando, de acôrdo com o caso, ácido fosfórico 0,3M ou hidróxido de sódio 0,2M. Na medida do pH utilizamos um potenciômetro Photovolt mod. 100, provido de eletródio de vidro. As soluções assim preparadas foram mantidas a 37°C e amostradas após 1 hora, 24 horas, 1 semana e 3 semanas do início da reação.

As amostras foram dialisadas contra dois litros de água durante 48 horas (1 litro para cada 24 horas), e em seguida contra os mesmos volumes de tampão por mais de 48 horas, utilizando-se o tampão da última diálise para a realização das eletroforeses[4]. O tampão de fosfato apresentava as seguintes características: pH 7,3, resistência ohmica 124 e concentração iônica 0,1. A resistência foi determinada numa ponte de condutividade eletrolítica portátil Leeds & Northup.

As eletroforeses foram realizadas em aparêlho Tiselius mod. 38 da Perkin Elmer Co., com células de 2 ml. e uma intensidade de corrente de 19,5 mA.

RESULTADOS

A reação das proteínas do sôro humano com o formaldeído é, como seria de esperar, fortemente influenciada pelo pH.



Em pH 7, observamos que os testemunhos permanecem inalterados, apresentando as quatro frações obtidas com o sôro original (A, α, β, γ). As mobi-

[3] Estas soluções foram esterilizadas em filtros Seitz esterilizantes (EK).

[4] As amostras após a diálise foram diluídas com volumes iguais de tampão.

lidades destas frações não sofrem alteração com o decorrer do tempo, resultado êste que está de acôrdo com a estabilidade que se esperaria do sôro em pH 7 e a 37°C. Nesse pH verificamos modificações sensíveis nos diagramas eletroforéticos obtidos com as amostras tratadas pelo formaldeído (fig. 1).

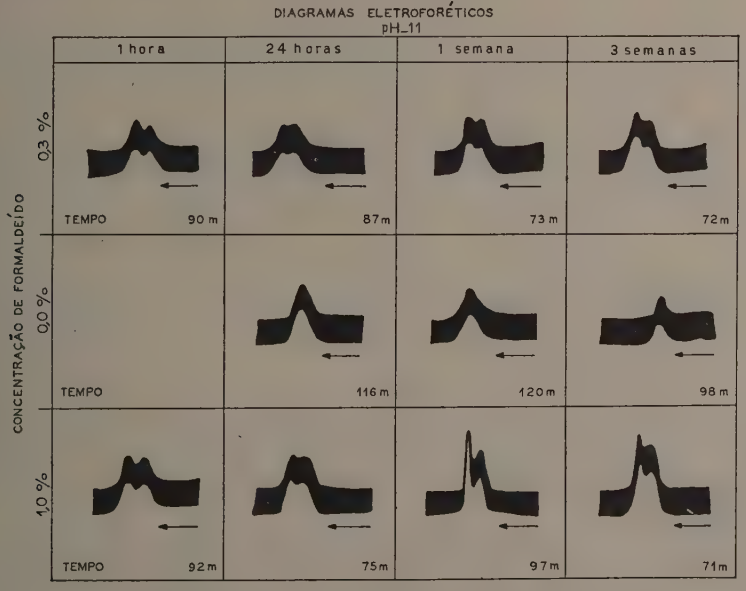
Os diagramas caracterizam-se por apresentarem — após 1 hora para a solução contendo 1% de formaldeído e após 24 horas para àquela contendo 0,3% — sòmente duas frações cujas mobilidades aumentam com o decorrer do tempo e com a concentração de formaldeído (Tabela I).

Verifica-se também um aumento da fração de menor mobilidade (globulinas) acompanhada da diminuição daquela de maior mobilidade (albuminas).

TABELA I
pH 7

Conc. de F	TEMPO DE REAÇÃO															
	1 hora				24 horas				1 semana				3 semanas			
	A	α	β	γ	A	α	β	γ	A	α	β	γ	A	α	β	γ
0,0%					5,0	3,7	2,7	0,8	4,7	3,9	2,8	0,8	5,0	3,7	2,6	0,7
0,3%	6,7		3,7	1,3	7,0		4,6		7,4		5,1		7,3		5,1	
1,0%	6,5		4,3		6,8		4,7		8,1		5,9		8,4		6,0	

As mobilidades são dadas em 10^{-5} cm². volt. $^{-1}$s.$^{-1}$.



A reunião das globulinas com formação de uma única fração, já havia sido observada por Hirtz (3), por Straub, Viollier & Müller (1) e por Perlman & Kaufman (5). Êste fato pode ser explicado levando-se em conta que o formal-

deído é capaz de formar ligações intermoleculares (pontes metilênicas) entre as globulinas. Esta explicação encontra apôio nos trabalhos de FRAENKEL-CONRAT & MECHAM (6). Êstes autores mostraram que o formaldeído, entre os pH 3 e 8, pode formar pontes metilênicas intermoleculares e concomitante aumento do pêso molecular médio das proteínas.

Poderíamos explicar por um mecanismo idêntico, a diminuição das albuminas e aumento das globulinas, havendo entretanto neste caso incorporação de uma parte das albuminas à fração globulínica.

Contribui, a favor desta hipótese, o fato dos testemunhos não apresentarem qualquer modificação e o de haver um aumento da velocidade de reação em conseqüência da maior concentração de formaldeído.

TABELA II
pH 11

Conc. de F	TEMPO DE REAÇÃO															
	1 hora				24 horas				1 semana				3 semanas			
	A	α	β	γ	A	α	β	γ	A	α	β	γ	A	α	β	γ
0,0%					4,6				4,8				4,6			
0,3%	5,6	4,1			6,6	5,5			8,3	6,4			8,5	6,5		
1,0%	5,9	4,0			7,5	5,9			7,8	6,0			8,0	6,2		

As mobilidades são dadas em 10^{-5} cm². volt. $-1_{s.}-1$

Observamos que os testemunhos de pH 11, quando dialisados contra a água, formam um precipitado que também não é solúvel no tampão de fosfato usado na eletroforese.

A análise eletroforética do sobrenadante mostrou a existência de apenas uma fração com mobilidade que corresponde ao componente que se desloca mais rápido. Dois fatos sugerem ser esta fração constituída pela albumina: a sua mobilidade e o fato da albumina de plasma bovino cristalizada não precipitar nestas mesmas condições experimentais (4).

A presença do precipitado sòmente nos testemunhos indica haver uma ação solubilizante do formaldeído sôbre as globulinas, evitando que as mesmas precipitem quando dialisadas.

A reação em pH 11 parece ser bastante rápida, pois, com apenas 1 hora de reação o diagrama eletroforético só nos mostra duas frações, tanto na solução contendo 1% de formaldeído como naquela com 0,3% (fig. 2). Com o passar do tempo nota-se ainda um aumento pronunciado na mobilidade dessas duas frações (Tabela II).

Neste pH há também agrupamento das globulinas em uma fração única que ainda neste caso parece ser devida à formação de pontes metilênicas entre as globulinas pela ação do formaldeído. Êste aldeído seria também o responsável pelo deslocamento do ponto isoelétrico e conseqüente aumento das mobilidades graças a sua combinação com os grupamentos básicos das proteínas.

TABELA III
pH 3

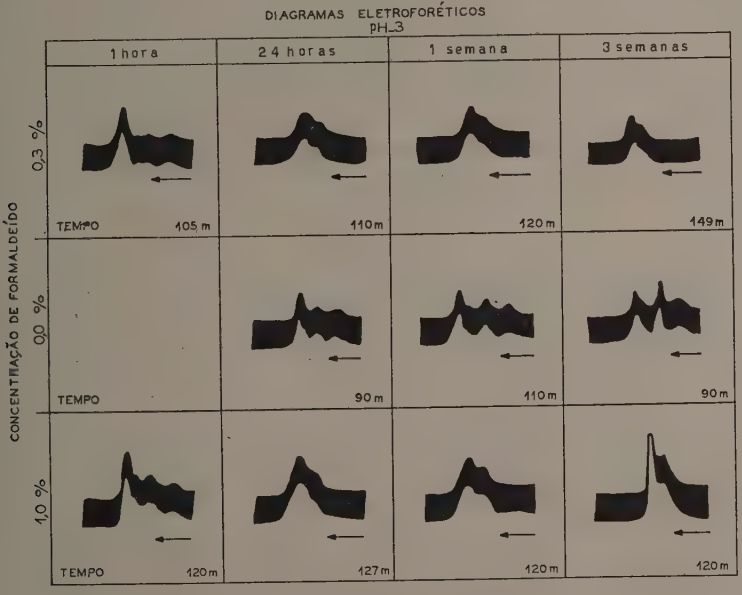
Conc. de F	TEMPO DE REAÇÃO															
	1 hora				24 horas				1 semana				3 semanas			
	A	α	β	γ	A	α	β	γ	A	α	β	γ	A	α	β	γ
0,0%					5.4	4,5	3,0	0,8	5,6	4,7	2,9	0,9	5,2	4,4	2,7	10,
0,3%	5,4	4,2	3,2	0,7	4,5	2,9	0,7		4,0	2,9			4,0	2,9		
1,0%	5,3	4,2	3,1	0,6	4,5	3,1			4,6	3,2			4,7	3,1		

As mobilidades são dadas em 10^{-5} cm.^{2}. volt.$^{-1}$s.$^{-1}$

Em pH 3 verificamos que, sòmente após três semanas, os testemunhos apresentam modificação no diagrama eletroforético, havendo então diminuição das albuminas e aumento da fração correspondente a β globulina (fig. 3).

O estudo das soluções em pH 3 e contendo formaldeído, mostra que, depois de 24 horas de reação, há alteração nos diagramas eletroforéticos, notando-se em ambas as concentrações de formaldeído usadas, uma diminuição das mobilidades das frações existentes. Com uma semana a reação pràticamente se estabiliza, observando-se então em ambas as concentrações apenas duas frações[5] (Tabela III).

A diminuição das mobilidades das frações eletroforéticas observadas em pH 3 poderia ser explicada pelo aumento do ponto isoelétrico das referidas



[5] As amostras de pH 3 contendo formaldeído e retiradas após 1 hora de reação, quando dialisadas contra água apresentaram um precipitado que mostrou-se, entretanto, solúvel no tampão de fosfato (pH 7,3).

frações, aumento êste que seria devido a desnaturação das proteínas e conseqüente liberação de grupamentos básicos, devido à rutura das ligações de hidrogênio. Esta explicação estaria de acôrdo com os trabalhos de PUTMAN & NEURATH (7), quando os mesmos observaram uma diminuição na mobilidade de uma das frações obtidas pelo tratamento da albumina de sôro bovino com o cloridrato de guanidina.

Tendo em vista que os testemunhos não apresentam qualquer modificação nas mobilidades eletroforéticas, poderíamos concluir que seria o formaldeído o responsável pela desnaturação e subseqüente aumento do ponto isoelétrico. Se considerarmos, entretanto, que a reação do formaldeído com as proteínas é mínima nesse pH (4), e se levarmos em conta os trabalhos de FRAENKEL-CONRAT & MECHAN (6) torna-se difícil aceitarmos tal explicação.

Julgamos, portanto, que sòmente um estudo pormenorizado da reação nesse pH poderá permitir uma interpretação segura do comportamento do sôro humano em pH 3.

Agradecimento — Agradecemos ao Dr. A. CURY e ao Sr. P.C.L. PORTELADA, do Laboratório de Microbiologia da Faculdade Nacional de Farmácia, a esterilização das soluções testemunhos.

SUMMARY

The effect of formaldehyde on the proteins of human serum (at 37°C and buffered at pH 3, 7 and 11) was followed by electrophoresis.

At pH 7 and 11 only two fractions with increased mobilities were observed after 24 hours of reaction. The slower fraction probably results from the grouping of the globulins through intermolecular cross-linking.

The appearance of only two fractions is faster, and the increase in mobilities is more pronounced, at high pH and formaldehyde concentration.

The changes observed at pH 3 are difficult to interpret and were, probably, not due to the action of formaldehyde.

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Rev. Brasil. Biol., 15 (3) : 271-277
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A SYNOPSIS OF THE COCKROACHES OF THE BRAZILIAN GENUS "BIONOBLATTA" (Orthoptera, Blattidae) [1]

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(With 9 text-figures)

The genus *Bionoblatta* Rehn was based on a single species, *mastrucata* (Rehn). Thanks to a loan of specimens generously arranged by Dr. José C. M. Carvalho, of the Museu Nacional, Rio de Janeiro, it is now possible to refer to *Bionoblatta* two species earlier described in other genera but which show very close agreement with the original generic diagnosis. The geographic distribution of *Bionoblatta* is reviewed and a key to the three known species presented.

Comparison of specimens with photographs (figs. 8 and 9 of this paper, and fig. 29 of Rehn, 1937) should permit the recognition of this distinctive genus. *Bionoblatta* belongs in a group usually treated as the Perisphaerinae, in the complex of genera centering about *Hormetica*. Recently, J. W. H. Rehn (1951, pp. 83-86) has applied the subfamily name Brachycolinae to *Brachycola* and *Hormetica*, genera including fully winged species.

In the absence of a needed generic revision, two papers by Rehn (1930, 1937) will help a great deal to distinguish the genera likely to be confused with *Bionoblatta*. Unlike *Bionoblatta*, *Hormetica* has overlapping tegmina, which may be fully developed or abbreviated in length. In *Parahormetica* the dorsal surface of the tegmina and abdomen varies from smooth to punctate, not shagreened or heavily rugose as in *Bionoblatta*. The tegmina of *Bionoblatta* are broadly rounded apically, and are more or less ovate, but those of *Parahormetica* are narrowed apically and usually are roughly triangular. Habitus illustrations of *Parahormetica* were given by Saussure (1864, fig. 35) and Costa Lima (1939, fig. 101).

Bionoblatta is known only from southern Brazil, in and near the State of Minas Gerais.

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[1] Received for publication June 13, 1955.

Bionoblatta Rehn

Bion Rehn, Trans. Amer. Ent. Soc., 63 : 252,1937. (Preoccupied)
Bionoblatta Rehn, Ent. News, 51 : 60, 1940. (N. name for *Bion*, preocc.).

Genotype: *Bion mastrucatus* Rehn, monobasic.

Study of the two species found to be congeneric with the genotype shows that only minor modifications of the original generic description are required. In *itatiayae* (Miranda Ribeiro) the tegmen extends over a portion of the second abdominal tergum, instead of reaching only to the first tergum. In both *itatiayae* and *diabola* (Saussure) the front femur has a genicular spine as well as a spine on the ventro-posterior margin. These spines are small, inconspicuous, and if broken are represented only by small pits. Fig. 3 illustrates the small arolium which is characteristic of *Bionoblatta*.

KEY TO THE SPECIES OF *BIONOBLATTA*

1.	Entire dorsal surface of abdomen brown ochre (fig. 8) ...	*itatiayae* (Miranda Ribeiro)
—	Dorsal surface of abdomen black or banded with yellowish red.	2
2.	Dorsal surface of abdomen black, with a broad median longitudinal band of yellowish red (fig. 9).	*diabola* (Saussure)
—	Dorsal surface of abdomen blackish except for narrow lateral borders and supra-anal plate, which are reddish.	*mastrucata* (Rehn)

Bionoblatta mastrucata (Rehn)

Bion mastrucatus Rehn, Trans. Amer. Ent. Soc. 63 : 253-256, figs. 18, 22, 29, 1937.

The unique type, in the Museu Paulista, São Paulo, was collected in the Serra do Itaculumy, and Rehn (1937) mistakenly ascribed this locality to the State of São Paulo, Brazil. Dr. Carvalho has explained that Itaculumy occurs instead in the State of Minas Gerais. Itaculumy is a mountain rising to a height of 5,896 feet on the southern outskirts of Ouro Preto, about 45 miles southeast of Belo Horizonte and about 170 miles directly north of Rio de Janeiro. No specimens of *mastrucatus* have been examined.

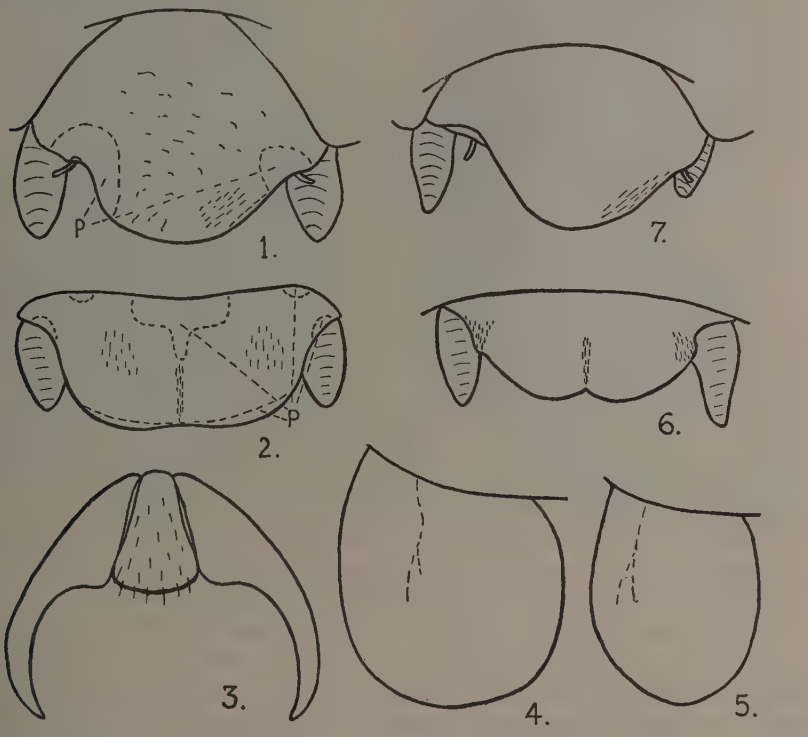
Bionoblatta diabola (Saussure) comb. n.

(Figs. 1-4, 9)

Brachycolla diabolus Saussure, Revue et Mag. Zool. (2), 16 : 344-345, 1864; Rehn, Trans. Amer. Ent. Soc. 63 : 253, 1937.
Hormetica diabolus Saussure, Mem. Hist. Nat. Mex. 4 : 182-183, 1864; Miss. Sci. Mex. et Amer. Cent., Pt. 6 : 99, 1870.

Specimen examined: São José do Barreiro, Serra da Bocaina, São Paulo, Brazil, 1,500 meters (about 4,800 ft.) alt., March 3, 1953 (Giselle Machline, collector) 1 male (Museu Nacional, Rio de Janeiro).

This species was originally described simply from "Brasilia". In the later 1864 paper, however, SAUSSURE indicated that the type, apparently unique, is on deposit in the Paris Museum, and that it was collected by Augustin F.C. de SAINT-HILAIRE (1779-1853), a renowned botanist who traveled extensively in Brazil, especially in Minas Gerais and nearby States. In the absence of exact evidence on the Brazilian locality from which the type of *diabola* was obtained, and in view of the apparently restricted distribution of this roach, we suspect that the species may be so localized that the type locality is near the area from which the specimen here described was taken.



Bionoblatta diabola (Saussure), male. — Fig. 1: Ventral view of subgenital plate (P = pale areas); fig. 2: dorsal view of supra-anal plate; fig. 3: claws and arolium of left hind tarsus, exterior view; fig. 4: left tegmen, dorso-lateral view. *Bionoblatta itatiayae* (Miranda Ribeiro), male — Fig. 5: Left tegmen, dorso-lateral view; fig. 6: dorsal view of supra-anal plate; fig. 7: ventral view of subgenital plate.

São José do Barreiro is a small town on the northern margin of the Serra da Bocaina in the extreme eastern portion of the State of São Paulo, about 80 miles west and slightly north of Rio de Janeiro. MACHLINE probably collected this specimen in the mountains, the higher peaks of which reach 6,792 feet. SAINT-HILAIRE (1833, Book 1, pp. 106-107) wrote of seeing the area along the Parahyba (Paraiba) River and referred to the Serra da Bocaina, in connection with his Brazilian travels, so it is conceivable that the type was collected there.

The descriptive notes in SAUSSURE's second 1864 paper are more complete than the original description. In particular, he mentioned a wide reddish band on the abdomen, bearing whitish warts, and two black apical segments of the abdomen. SAUSSURE's measurements are nearly identical to those of the present specimen, and, considering the structural and color features of the description, leave no doubt as to the identification here reported.

Descriptive notes: Head with face and vertex deeply punctate; interocular distance noticeably greater than distance between antennal sockets (ratio of 50 to 43); antenna with basal 12 segments polished, beyond segment 12 unpolished; tegmen subcircular, extending midway over first abdominal tergum, mesal portion of apical margin appearing oblique when specimen is seen in dorsal view (fig. 9), but scarcely oblique when viewed dorso-laterally (fig. 4); tegmina separated by a distance equal to one-third the width of a single tegmen; wing evident beneath tegmen, vestigial but reaching nearly to apex of tegmen; exposed portions of mesonotum and metanotum smooth in anterior two-thirds except for sparse deep punctures, a slightly raised longitudinal median ridge about half the length of each segment, posterior third with delicate transverse wrinkles; abdomen heavily rugose above, the median half of terga 1-7 (which is pale in color) with low, wartlike protuberances; latero-posterior angles of terga 2-5 rounded, of terga 6-7 slightly produced but decidedly obtuse; supra-anal plate (fig. 2) transverse, posterior margin weakly and broadly emarginate, surface delicately marked with wrinkles, shallowly concave near middle of each lateral half and along median line in posterior half; subgenital plate (fig. 1) moderately asymmetrical, weakly and sparsely punctate, with shallow elongate impressed lines as illustrated, the whole organ convex except for a broad submarginal concavity on left side.

Coloration: Dorsal surface black except for median area of abdomen; posterior half of tergum 1 and entire length of terga 2-9 with a yellowish red median area extending onto supra-anal plate as shown by dotted areas (fig. 2, *p*); supra-anal plate with narrow yellow subapical margin and two spots on each side near base of cercus; main pale area of abdomen dull reddish (beige) near median line, yellowish laterally, and very pale clay yellow on warts. Antennae black; face with two dirty white dots, subequal in size, mesal from each antennal socket; eyes pale brown; subgenital plate with yellowish spots as illustrated (fig. 1, *p*); styli reddish brown; pulvilli of tarsi whitish; remainder of ventral surface and legs black.

Measurements (in millimeters) — Length: body, 36; pronotum, 13.7; tegmen, 10.5; posterior tibia, 9.6; greatest width: pronotum, 16; tegmen, 9; abdomen, 18.

Bionoblatta itatiayae (Miranda Ribeiro) comb. n.

(Figs. 5-8)

Hormetica itatiayae Miranda Ribeiro, Rev. Mus. Paulista 20 : 217-219, Fig. 1, 1936.

Specimen examined: Monte Serrat, Serra do Itatiaya, Brazil (Dr. Bruno Lobo, collector), 1 male, one of 4 original cotypes, here selected as lectotype. (Museu Nacional, Rio de Janeiro).

Fig. 8 — *Bionoblatta itatiayae* (Miranda Ribeiro), male, dorsal view. Fig. 9 — *Bionoblatta diabola* (Saussure), male, dorsal view. (Photographs from the Smithsonian Institution).

The type locality, Itatiaya, is in a mountain area just west of Resende, about 90 miles northwest of Rio de Janeiro. The summit marks the boundary between the States of Minas Gerais and Rio de Janeiro, with the boundary of São Paulo a very short distance further west, so the locality is practically at the junction of the three States (Holt, 1928, pp. 253, 316). Monte Serrat is in the State of Rio de Janeiro, and it is a prominence attaining an altitude of about 3,000 feet above sea level on the slopes of Itatiaya. Rainforest conditions occur at the higher altitudes. Holt (1928) has discussed and illustrated the general conditions and life zones of Itatiaya in considerable detail.

Because Miranda Ribeiro's figure of the supra-anal plate of *itatiayae* indicates a more pronounced emargination of the posterior margin than is shown by the specimen examined, I have made a sketch of this structure (fig. 6).

Descriptive notes: Head with face and vertex punctate about as in *diabola;* interocular distance scarcely less than distance between antennal sockets (ratio of 35 to 37); antenna with basal 11 segments polished, beyond segment 11 unpolished; tegmen suboval, extending over basal part of second abdominal tergum; tegmina separated by a distance equal to about two-thirds the width of a single tegmen; wing evident beneath tegmen, vestigial and somewhat shorter than tegmen; exposed portions of mesonotum and metanotum as in *diabola* except that a median longitudinal ridge extends across both segments; abdomen heavily rugose above, the median third of terga 1-7 with low, wartlike protuberances; latero-posterior angles of terga 2-7 obtuse, well rounded; supra-anal plate (fig. 6) with posterior margin decidedly emarginate; subgenital plate (fig. 7) asymmetrical, inconspicuously and sparsely punctate, decidedly convex except for submarginal concavity on left side.

Coloration: General color black except abdomen; pronotum with narrow indistinct yellowish submarginal spot just mesad of recurved margin on each side about one-third distance from middle of front margin to latero-posterior angle; median portion of anterior recurved margin of pronotum, higher tuberculate prominences of pronotal disc (horseshoe shaped, extending posteriorly from main "boss" on each side, closely posteriorly), principal rugosities within "horseshoe," median portion of mesonotum and metanotum, and lateral and apical margins of tegmina plainly tinged with Indian red; abdomen brown ochre, warts whitish yellow; supra-anal plate yellow ochre; cerci brownish black; face with 2 yellow ochre dots mesal from each antennal socket, the more ventral of the two dots twice as large as the upper one; eyes yellow ochre; general color of venter of abdomen brown ochre; sterna 6-7, posterior margin of subgenital plate, and styli darkened, Van Dyke brown; legs spotted on knees and on coxal and trochanteral articulations with burnt sienna; pulvilli of tarsi grayish white; remainder of ventral surface and legs dark blackish brown.

Measurements (in millimeters) — Length: body, 29; pronotum, 11; tegmen, 8.5; posterior tibia, 9; greatest width: pronotum, 13.5; tegmen, 6; abdomen, 17.

DISCUSSION

In view of the close relationship of the three species of *Bionoblatta,* evidenced especially by the sculptured body surface, it is instructive to consider briefly the probable isolating factors which may have contributed to speciation trends in these flightless, short-legged, and presumably somewhat clumsy roaches. Although the known specimens of *diabola* and *itatiayae* were not taken near the highest elevations of their respective mountains, the captures occurred a considerable distance above the floor of the Paraiba River. The Serra do Itatiaya is only about 25 miles north of the Serra da Bocaina at the nearest point, but not only do the river and the valley, reduced to less than 10 miles in width in certain places, probably constitute an effective natural barrier to

the mingling of these populations, but the two mountains represent distinct major mountain systems, which bear the general terms Serra da Mantiqueira and Serra do Mar. The Serra do Itaculumy, habitat of *mastrucata,* is about 160 miles northeast of Itatiaya, and includes a distinctive peak at the eastern margin of the Serra da Mantiqueira. If more intensive collecting should establish the fact that the species of *Bionoblatta* are restricted to the vicinity of these higher mountains within the upland area of this region of Brazil, as is suggested by present information, then the evolution of the species probably has been correlated with the geological history of these mountains. It is quite possible that additional species of *Bionoblatta* will be found associated with other distinctive mountains nearby, especially Pico da Bandeira located about 100 miles east of Itaculumy.

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Setembro, 1955 — Rio de Janeiro, D. F.

NOTA SÔBRE A FAUNA HELMINTOLÓGICA DE RÉPTEIS BRASILEIROS [1]

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(Com 18 figuras no texto)

Recentemente tivemos oportunidade de autopsiar alguns répteis que nos forneceram interessante material helmintológico, do qual destacamos algumas espécies para serem apresentadas na presente nota. Assim, referimos *Oswaldocruzia subauricularis* (Rudolphi, 1819) e *Falcaustra mascula* (Rudolphi, 1819) em novos hospedadores e descrevemos uma nova espécie pertencente ao gênero *Hastospiculum* Skrjabin, 1923: *Hastospiculum digiticaudum* sp.n.

Oswaldocruzia subauricularis (Rudolphi, 1819)
(Figs. 1-4)

Hospedador — *Enyalius catenatus* (Wied).

Proveniência — Floresta da Tijuca, Rio de Janeiro, D.F., Brasil.

Esta espécie, parasita comum de batráquios, foi observada por TRAVASSOS nos seguintes hospedadores: *Ceratophrys cornuta* (L.), *Bufo marinus* (L.), *Bufo crucifer* Wied, *Hyla faber* Wied, *Hyla mesophaea* Hens. e *Phyllomedusa burmeisteri* Begr.

Ao assinalarmos agora sua presença no intestino delgado de um lagarto, pertencente à família *Iguanidae,* representamos alguns aspectos do macho e referimos as seguintes medidas: comprimento da bolsa copuladora — 0,28 a 0,33 mm; largura da bolsa copuladora — 0,45 a 0,53 mm; distância entre a origem dos raios dorsais externos e a divisão do raio dorsal — 0,063 mm; comprimento dos espículos — 0,24 a 0,25 mm.

O material estudado está depositado na coleção helmintológica do Instituto Oswaldo Cruz sob o n.º 20.792 a-c.

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[1] Recebido para publicação a 17 de maio de 1955.

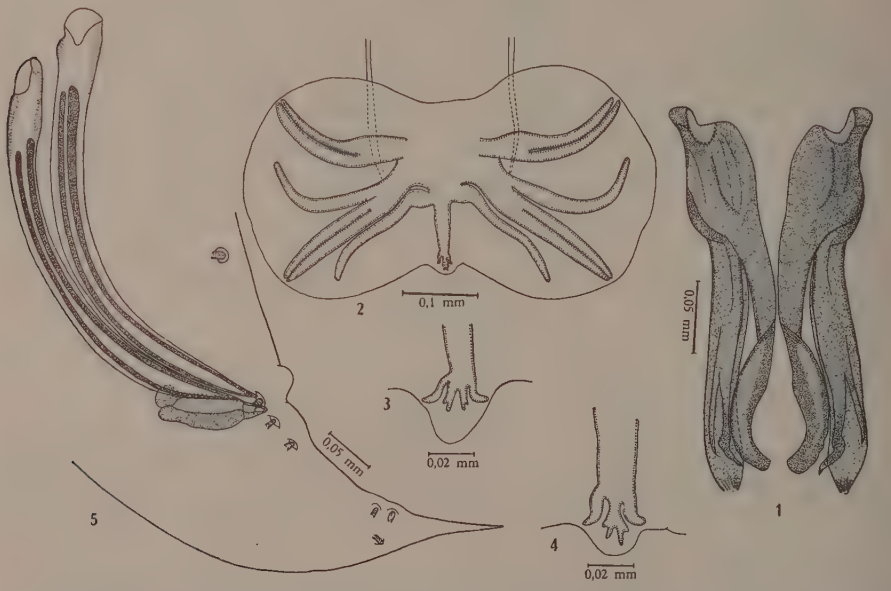
Falcaustra mascula (Rudolphi, 1819)
(Fig. 5)

Hospedador — *Leimadophis poecilogyrus* (Wied).

Proveniência — Rio de Janeiro, D.F., Brasil.

Esta espécie, parasita de ofídios e de batráquios, foi já assinalada nos seguintes hospedadores: *Eudryas bifossatus* (Raddi), *Coluber lichtensteinii* Wied, *Pseudophis bivittatus* Fitz., *Elosia nasus* (Licht.), *Hyla faber* Wied, *Bufo paracnemis* Lutz, *Leptodactylus ocellatus* (L.) e *Leptodactylus pentadactylus* (Laur.).

Ao assinalarmos agora sua presença no intestino delgado de uma outra espécie de ofídio, representamos a cauda do macho e referimos os seguintes caracteres: 3 pares de papilas pré-anais e 6 pares pós-anais presentes; espículos sub-iguais, com 0,40 e 0,43 mm de comprimento; gubernáculo com 0,096 mm de comprimento; anus situado a 0,23 mm da ponta da cauda; ventosa pouco desenvolvida, situada a 1,58 mm da extremidade posterior do corpo.



Oswaldocruzia subauricularis (Rudolphi, 1819) — Fig. 1: Espículos; fig. 2: bolsa copuladora; figs. 3 e 4: terminação do raio dorsal. *Falcaustra mascula* (Rudolphi, 1819) — Fig. 5: Cauda do macho, vista lateral.

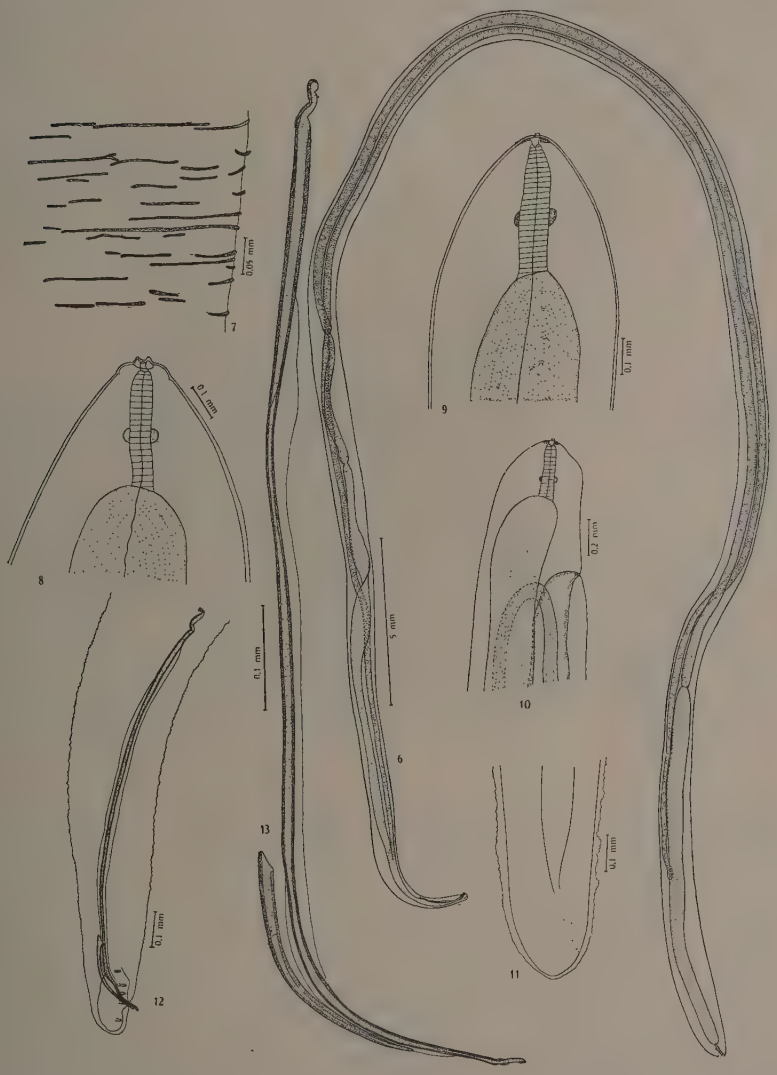
O material que examinamos está depositado na coleção helmintológica do Instituto Oswaldo Cruz sob o n.º 20.819.

Hastospiculum digiticaudum sp. n.
(Figs. 6-18)

Comprimento — Machos 38,59 a 53,17 mm; fêmea 76,88 mm.

Largura — Machos 0,57 a 0,78 mm; fêmea 0,80 mm.

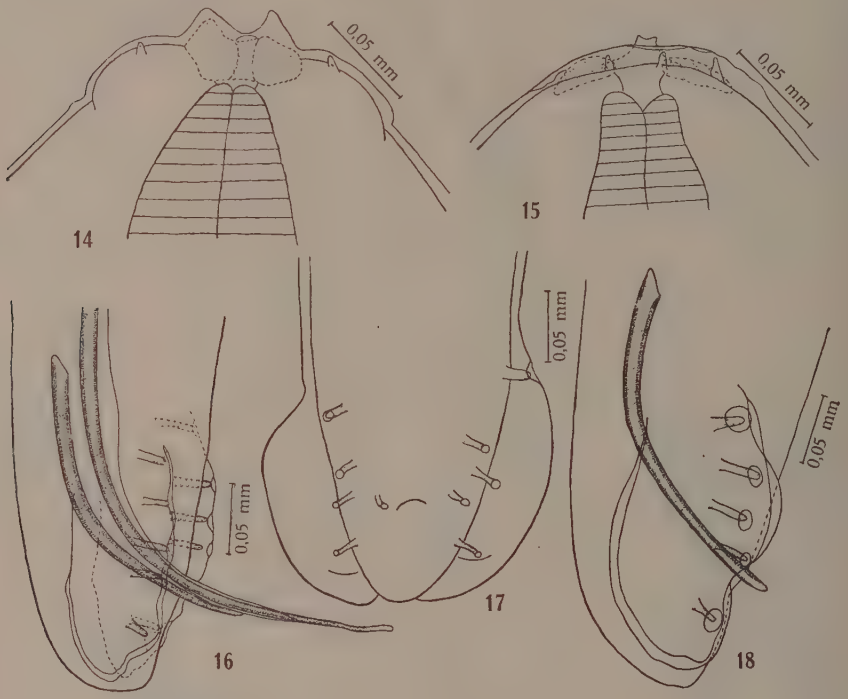
Corpo de coloração branca em vida, com a extremidade posterior afilada e com cutícula estriada transversalmente e provida de espessamentos quitinisados



Hastospiculum digiticaudum sp.n. — Fig. 6: Macho, total; fig. 7: espessamentos cuticulares, na região esofagiana do corpo; fig. 8: extremidade anterior do macho, vista dorsal; fig. 9: extremidade anterior do macho, vista lateral; fig. 10: extremidade anterior da fêmea, vista lateral; fig. 11: extremidade posterior da fêmea, vista lateral; fig. 12: cauda do macho, vista lateral; fig. 13: espículos. (Figs. 6, 8 e 12 do holótipo; fig. 7 do parátipo n.º 20.794 b; figs. 9 e 13 do parátipo n.º 20.794 a; figs. 10 e 11 do alótipo).

salientes, irregulares na forma e na disposição; êsses espessamentos emprestam ao corpo um aspecto espinhoso. Extremidade anterior com duas pequenas dila-

tações laterais em forma de ombreiras, cada uma possuindo 3 papilas; existem, assim, 6 papilas cefálicas: um par látero-dorsal, 1 par lateral e 1 par látero-ventral. Essas dilatações se projetam anteriormente, constituindo duas saliências labiais que medem 0,021 a 0,034 mm de comprimento nos machos e 0,017 mm



Hastospiculum digiticaudum sp.n., macho — Fig. 14: Extremidade cefálica, vista dorsal; fig. 15: extremidade cefálica, vista lateral; fig. 16: cauda, vista lateral; fig. 17: cauda, vista ventral; fig. 18: cauda, vista lateral. (Figs. 14 e 18 do holótipo; figs. 15 e 16 do parátipo n.º 20.794 a; fig. 17 do parátipo n.º 20 794 b).

na fêmea. Bôca alongada no sentido dorso-ventral, circundada lateralmente pelas ombreiras e lábios, e dorsal e ventralmente por porções espessadas da cutícula. Cavidade bucal pequena. Esôfago com 6,90 a 9,48 mm de comprimento nos machos e 9,01 mm na fêmea; é dividido em duas porções; uma anterior, curta, mais delgada, muscular, e outra posterior, longa, mais larga, glandular. A porção muscular do esôfago mede nos machos 0,23 a 0,33 mm de comprimento por 0,08 mm de largura e na fêmea 0,33 mm por 0,10 mm. A porção glandular do esôfago mede nos machos 6,70 a 9,21 mm de comprimento por 0,27 a 0,43 mm de largura e na fêmea 8,71 mm por 0,30 mm. Intestino mais ou menos retilíneo. Anel nervoso situado a 0,22 a 0,23 mm da extremidade anterior nos machos e a 0,25 mm na fêmea. Papilas cervicais e poro excretor não evidenciados.

Fêmea didelfa, anfidelfa, com vulva de lábios não salientes situada a 0,73 mm da extremidade anterior. Ovejetor dirigido para trás, medindo 1,91 mm de comprimento. Úteros não contendo ovos. Anus atrofiado. Ápice caudal arredondado.

Machos com espículos desiguais e dissemelhantes: o espículo maior, alado, mede 0,97 a 1,13 mm de comprimento; o espículo menor mede 0,23 a 0,25 mm de comprimento. A relação espicular varia de 1 : 4,2 a 1 : 4,6. Tubo genital dirigido para diante; o testículo termina ao nível da região média da porção glandular do esôfago. Anus situado a 0,07 a 0,09 mm do ápice caudal, que é arredondado. Cauda provida de asas laterais espessadas, que não envolvem o ápice e que são sustentadas por 5 pares de papilas pedunculadas, delgadas, assim distribuidos: 3 pares pré-, 1 par ad- e 1 par pós-anal.

Habitat — Cavidade geral de *Philodryas aestivus* (Schl.).

Proveniência — Restinga da Barra da Tijuca, Rio de Janeiro, D.F., Brasil.

Holótipo macho n.º 20.793 a, alótipo fêmea n.º 20.793 b e dois parátipos machos n.º 20.794 a-b, depositados na coleção helmintológica do Instituto Oswaldo Cruz.

Discussão — No gênero *Hastospiculum* são referidas as seguintes espécies: *H. varani* Skrjabin, 1923, (espécie tipo); *H. macrophallos* (Parona, 1889); *H. bipinnatum* (Linstow, 1899); *H. gouldi* Yorke & Maplestone, 1926; *H. spinigerum* Chandler, 1929; *H. setiferum* Chitwood, 1932; *H. onchocercum* Chitwood, 1932; *H. onchocercum major* Desportes, 1941 e *H.* sp. Johnston & Mawson, 1948. A espécie nova agora proposta se distingue logo de tôdas elas pelas asas caudais dos machos, que não envolvem o ápice caudal; a relação espicular torna-a mais próxima de *H. bipinnatum* (Linstow, 1899) e *H. gouldi* Yorke & Maplestone, 1926, das quais se distingue, também, pelas papilas caudais dos machos.

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Rev. Brasil. Biol., 15 (3) : 285-292
Setembro, 1955 — Rio de Janeiro, D. F.

EFFECT OF CARBON TETRACHLORIDE ON THE RAT LIVER XANTHINE OXIDASE [1]

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(With 5 text-figures)

It is a well known fact that the administration of carbon tetrachloride produces in the rat a fat infiltration and a center lobular necrosis of the liver. FORBES & NEALE showed that sodium xanthine and liver extracts containing xanthine are able to prevent the necrotic lesions when injected previously from the poison (7) (8). We were, therefore, interested to know if carbon tetrachloride had some effect on the xanthine oxidase and xanthine dehydrogenase of the rat liver. In a preliminary report we showed that this drug activated the enzyme *in vivo* and *in vitro* (21). Moreover, we studied the effect of sodium xanthine and cyanocobalamin (vitamin B_{12}), on xanthine oxidase and xanthine dehydrogenase, in normal and starved rats. In the present work these and other experiments are reported and the results discussed.

MATERIAL AND METHODS

Male white Wistar rats weighing 90 to 195 g were used throughout this study. All the animals were submitted to a well balanced diet. Carbon tetrachloride was injected subcutaneously in the dosage of 0.1 ml/100 g body weight.

The animals were killed by decapitation and the liver after being freed from blood and adjacent tissues was weighed and homogenized in a Warren blendor with five volumes of distilled water. The homogenates were incubated for a 40 minutes period at 37°C to minimize the endogenous respiration as indicated by AXELROD & ELVEHJEM. Xanthine oxidase was determined by the manometric method of AXELROD & ELVEHJEM (1). The high values observed for the endogenous respiration were avoided by the use of a pyrophosphate buffer pH 8.6 instead of a phosphate buffer pH 7.4 as proposed by DHUNGAT & SREENIVASAN (3). Each Warburg flask received 1.5 ml of the homogenate

Fig. 1 — Xanthine oxidase of normal rats (A) and rats starved 24 (B) and 48 hours (C).

corresponding to 250 mg of fresh tissue. Xanthine 0.05 M dissolved in NaOH 0.05 M was used as substrate and 0.2 ml was pipetted on to the side arm of the flasks. Into the central well 0.2 ml of 20% KOH was added with a folded filter paper. The total volume was completed to 3 ml in all flasks with the pyrophosphate buffer. The gas phase was air and the temperature of the water 37°C. After 10 minutes for the equilibration of the temperature in the flasks the 0 time was recorded and after 20 minutes the substrate was tipped. The readings were taken each 20 minutes up to 120 minutes. The endogenous respiration without added xanthine was subtracted from the values of the other flasks. The results were expressed in C. mm O_2/g of dry weight per 60 minutes (17).

The colorimetric determination of xanthine was performed by the method of LITWAK *et al.* (11). The homogenates were incubated at 37°C with constant stirring during 120 to 180 minutes and 1 ml of the aliquot was measured and deproteinized by the addition of 10 per cent sodium tungstate and 0.66 N sulfuric acid. The clear filtrates were mixed with known volumes of the Folin-Ciocalteu reagent and the blue color measured in a fotocolorimeter at 660 mμ.

The dehydrogenase activity was determined by the colorimetric method of VILLELA based on the reduction of the triphenyltetrazolium chloride to red

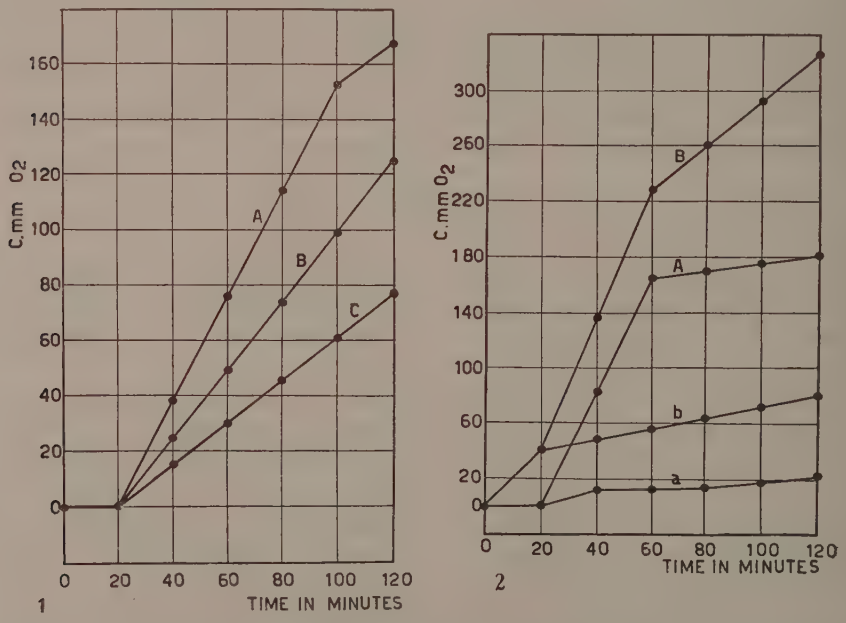

Fig. 1 — Xanthine oxidase of normal rats (A) and rats starved 24 (B) and 48 hours (C).. Fig. 2 — *In vitro* activation of xanthine oxidase by CCl_4. (A) rat liver homogenate. (B) homogenate added with CCl_4. (a) endogenous respiration. (b) endogenous respiration of the homogenate treated with CCl_4.

formazan in a evacuated Thunberg tube and the color extracted with petroleum ether (20). The results were expressed in microg of formazan reduced per mg dry weight of liver.

RESULTS

Liver of normal well fed rats presented values averaging 1638 ± 168 C mm O_2/g of dry weight per 60 minutes and rats starved for 24 and 48 hours, values of 1223 ± 32 C.mm and 921 ± 177 C.mm, respectively. The decrease of xanthine oxidase in starvation and in deficient protein diets was been reported by Litwak *et al.* (10) and by Westerfeld & Richert (23), which demonstrated the importance of some constituents of the diet to the synthesis of the enzyme. Fig. 1 shows the xanthine oxidase activity of liver homogenates of normal rats (A) and of rats starved for 24 hours (B) and 48 hours (C).

In vitro experiments were performed in 10 ml of the liver homogenates incubated with 0.1 ml of carbon tetrachloride for 15 minutes at 37°C. After centrifugation the bottom layer, free from CCl_4, was pipetted off and used for the measurements of the xanthine oxidase and dehydrogenase activities. The marked increase in the activity of the xanthine oxidase is clearly shown in the Fig. 2. It is interesting to note that the endogenous respiration was also markedly activated. However, xanthine dehydrogenase activity was slightly decreased.

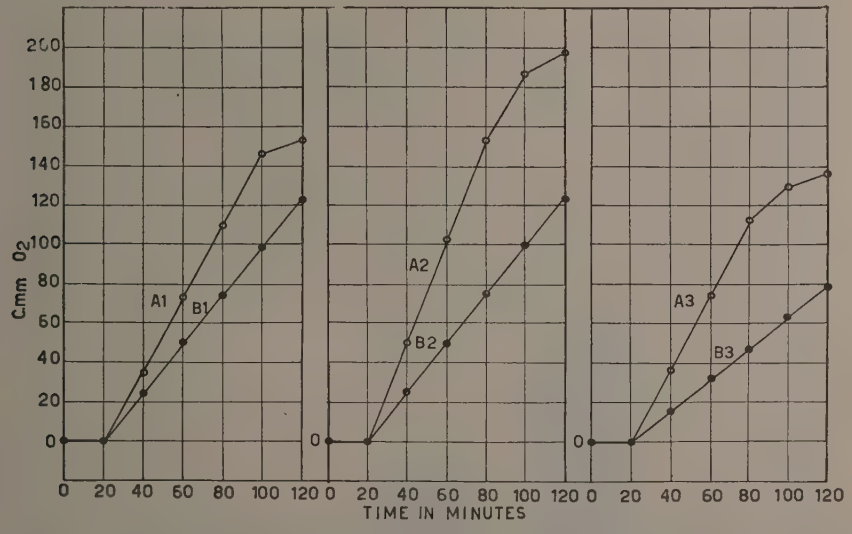

Fig. 3 — Liver homogenates xanthine oxidase of rats injected with CCl_4 and killed after 3 hours (A_1), 24 hours (A_2) and 48 hours (A_3), compared with non injected rats starved for 24 hours (B_1) (B_2) and 48 hours (B_3).

Rats submitted to a 24 hous starvation, injected subcutaneously with 0.1 ml carbon tetrachloride and killed 3 hours after the administration of the

drug showed a slight increase of the activity of the enzyme as compared with the controls. Small necrotic areas were visible in the liver of the former animals. The xanthine dehydrogenase presented normal values. When the rats were killed 24 and 48 hours after the administration of the poison all the livers showed fat infiltration and a typical necrosis so well described by CAMERON & KARUNARATNE (2). In these conditions the xanthine oxidase presented higher values chiefly in those rats starved for 48 hours. Xanthine dehydrogenase was decreased in these conditions. Fig. 3 summarizes these results.

POPPER et al. have shown that vitamin B_{12} (cyanocobalamin) when injected before the toxic dose of carbon tetrachloride is able to protect the rats from liver necrosis (16).

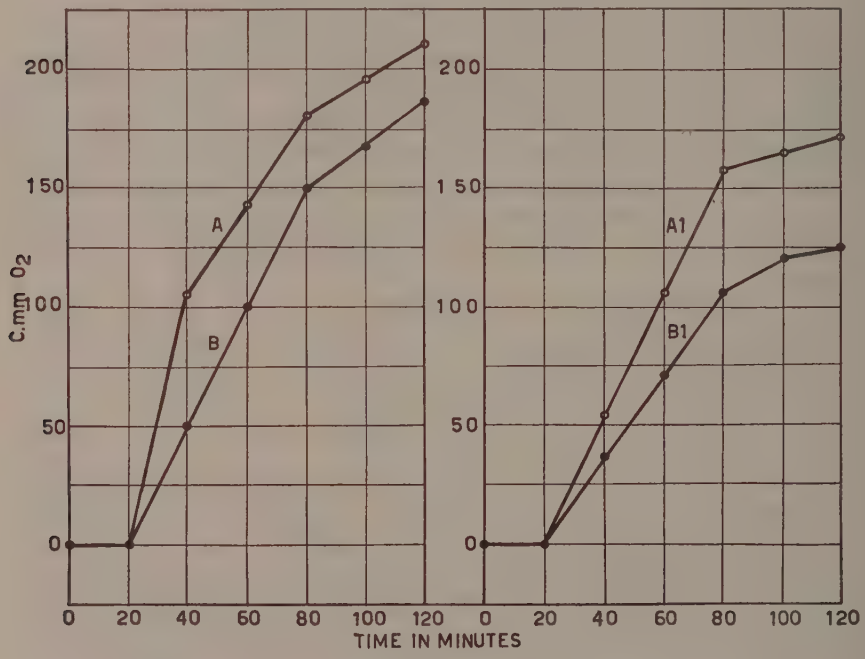

Fig. 4 — Xanthine oxidase of the liver of rats treated with vitamin B_{12} and injected with CCl_4 and killed 24 hours (A) and 48 hours (A_1) compared with rats injected with CCl_4 (B) (B_1).

We were interested to know if these substances have any effect on the xanthine oxidase and xanthine dehydrogenase of the liver in normal and poisoned rats. Sodium xanthine as well as vitamin B_{12} when injected to rats starved for 24 hours were ineffective on the xanthine oxidase activity. DIETRICH observed in rats given xanthine at a level of 150 mg/kg body weight for 23 consecutive days a slight higher activity for the xanthine oxidase as compared with non-treated controls (4), but these values, as pointed out by DIETRICH, were not significant. However, in rats receiving 100 mg/100 g body weight of

sodium xanthine before the toxic dose of carbon tetrachloride, we observed a 50 per cent inhibition of the xanthine oxidase and dehydrogenase activities of the liver. Similarly, vitamin B_{12} was unable to increase, significantly, the xanthine oxidase and xanthine dehydrogenase of the liver of normal rats except when carbon tetrachloride was injected 24 hours later (fig. 4).

A clear confirmation of the increase in the activity of the xanthine oxidase induced by carbon tetrachloride was obtained when the liver homogenates were incubated with the substrate and the xanthine measured colorimetrically by the method of LITWAK and coworkers (11). These results were recorded in Table I.

TABLE I

Oxidation of xanthine by liver homogenates in normal rats and rats treated with carbon tetrachloride measured colorimetrically

CONDITIONS	μg xanthine/g liver		
	Incubation time (minutes)		
	0	120	180
Normal	580	436	150
CCl_4 24 hr. *	314	8	0
CCl_4 48 hr. *	476	73	72
CCl_4 + vit. B_{12} 24 hr **	576	0	0
CCl_4 + vit. B_{12} 48 hr **	810	0	0

* Time of starvation after the injection of carbon tetrachloride (0.1ml/100g body-weight).
** Vitamin B_{12} (15 μg 24 hr. before the administration of carbon tetrachloride).

Some *in vitro* experiments were also performed with the liver enzyme prepared and purified by the technique of RICHERT, VANDERLINDE & WESTERFELD (18). The enzyme was extracted from the ammonium sulfate precipitates at pH 7.35. The activation of the xanthine oxidase by CCl_4 was followed in the

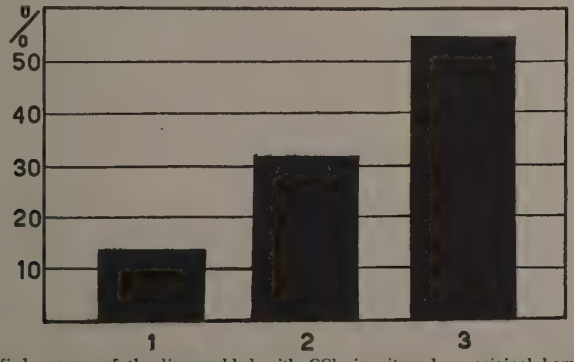

Fig. 5 — Purified enzyme of the liver, added with CCl_4 *in vitro*: 1 = original homogenate; 2 = first purification and 3 = final purification.

original homogenate and in the subsequent preparations obtained from 42-60% ammonium sulfate precipitates after a dialysis of 24 hours, against distilled water.

In all experiments 10 ml of the enzyme preparation was incubated with 0.1 ml of carbon tetrachloride during 15 minutes at 37°C and centrifuged. The results obtained seemed to show that the activation induced by CCl_4 is more apparent with the more purified preparations (fig. 5).

Other organic solvents like ethanol, ether and chloroform were tried in *in vitro* experiments to know its effect on the xanthine oxidase of rat liver. Some typical results are found in Table II.

TABLE II

CONDITIONS	C.mm 0^2/60 minutes	Per cent activation
Original homogenate	103.7	—
Plus ethanol	155.7	40.21
Plus ether	114.9	10.80
Plus chloroform	113.9	9.62

DISCUSSION

The activation of xanthine oxidase by carbon tetrachloride and other organic solvents could be compared with the activation by these solvents on the xanthine dehydrogenase of cow milk. Polonowsky *et al.* claimed that some organic solvents are able to break the lipoprotein linkage existing between the enzyme and the lipid of the milk and, therefore, increasing the activity of the enzyme (13) (14) (15). Similar mechanism could be only, partly accepted, for the xanthine oxidase of liver, since xanthine dehydrogenase is not activated. However, in experiments now in course on the effect of injected CCl_4, to the serum rat xanthine dehydrogenase it was found that a marked activation occurred after 24 hours from the administration of the poison. These facts seemed to show that the enzyme in blood serum is in different physico-chemical state than in the liver (12). It is interesting to recall that the mitochondria and other liver particulates are free of this enzyme, the xanthine oxidase being almost, exclusively, in the cell sap (22).

The protective effect of xanthine in carbon tetrachloride poisoned rats could be due to some inhibitory action of xanthine on the enzyme as was observed in *in vitro* experiments by Dixon & Elliott (6). Vitamin E deficiency has been shown by Dinning (5) and confirmed by Richert & Westerfeld (19) to increase the activity of xanthine oxidase. Since, liver necrosis is able to be protected by tocopherol (9), there is some possibility that the inhibition of

xanthine oxidase permits this effect. However, we were unable to obtain consistent results in rats fed with high vitamin E diets.

The inexpected effect produced by the administration of vitamin B_{12} in the activation of xanthine oxidase in poisoned rats suggests that the mechanism of the liver necrosis in this case should not be related to the activation of this enzyme.

SUMMARY AND CONCLUSIONS

1 — Liver xanthine oxidase was shown to be activated by carbon tetrachloride *in vitro* and *in vivo* and the results obtained by the authors showed to be statistically significant (21). The xanthine dehydrogenase activity in the same conditions was decreased slightly. Ethanol, chloroform and ether are also effective.

2 — Purified preparations of xanthine oxidase from rat liver were activated by carbon tetrachloride, chiefly in the more purified preparations of the enzyme.

3 — The activation by carbon tetrachloride is discussed as being due to the rupture of a lipoprotein complex of the enzyme and other cell constituents.

4 — Sodium xanthine when injected before a poisonous dose of carbon tetrachloride decreases markedly the xanthine oxidase and dehydrogenase activities of the liver.

5 — Vitamin B_{12} in the single dose of 15 microg when injected 24 hours before the poison increases the xanthine oxidase and dehydrogenase activities of the liver.

RESUMO

A xantina oxidase de fígado de ratos é ativada pelo tetracloreto de carbono *in vitro* e *in vivo* e os resultados obtidos pelos autores mostraram ser, estatìsticamente, significantes (21). A xantina desidrogenase, nas mesmas condições, tem sua atividade diminuida. Etanol, clorofórmio e éter são também ativadores.

Preparações purificadas de xantina oxidase de fígado de ratos foram também ativadas pelo tetracloreto de carbono.

A ativação da xantina oxidase pelo tetracloreto de carbono é explicada, como sendo devida a ruptura de um complexo lipoprotéico da enzima e outros constituintes celulares.

Xantina sódica quando injetada antes do tetracloreto de carbono diminui, sensìvelmente, tanto a atividade oxidase, como a atividade desidrogenase da enzima do fígado.

A vitamina B_{12}, quando injetada em dóse de 15 microgramas, 24 horas antes da administração de tetracloreto de carbono, aumenta as atividades da xantina oxidase e desidrogenase do fígado.

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STUDIES ON "AUSTRALORBIS CENTIMETRALIS".
I. Morphology, in comparison with "A. glabratus" [1]

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(With 28 text-figures)

In the city of Governador Valadares, situated in the eastern State of Minas Gerais, on terrane of the Archean system of the Rio Doce basin, four different planorbids may be found: two species of *Drepanotrema, Australorbis glabratus* (the most frequent), and a small species which is dealt with in the present paper. The four mentioned planorbids occur sympatrically in the little still-water baylets of the creek "Córrego do Bambú", a left affluent of the Rio Doce, wherefrom the specimens now studied were collected.

In his revision of the Brazilian planorbids, Lutz (1918) gave the name *Planorbis centimetralis* to the single species that he found in Minas Gerais, which was considered by him (Lutz, 1934) as the only transmitter of *Schistosoma mansoni* in that State. We know now, based on numerous publications by several authors, that another transmitting snail, *A. glabratus,* is widespread over a large zone of the State.

The occurrence of *A. centimetralis* in the region of Governador Valadares was mentioned by Penido, Pinto & Deslandes (1951), and by Pinto & Deslandes (1953). No reference exists to that region in Lutz's papers. We identified our specimens with *P. centimetralis* by comparison with Lutz's description (1918), and on account of some of them being found infected with *S. mansoni.* A justification for the placing of the species in the genus *Australorbis* will be published later.

A less trained observer is liable to mistake adult specimens of *centimetralis* for young *glabratus.* For this reason, at each step of the following description we will refer to the corresponding condition in young *glabratus*.

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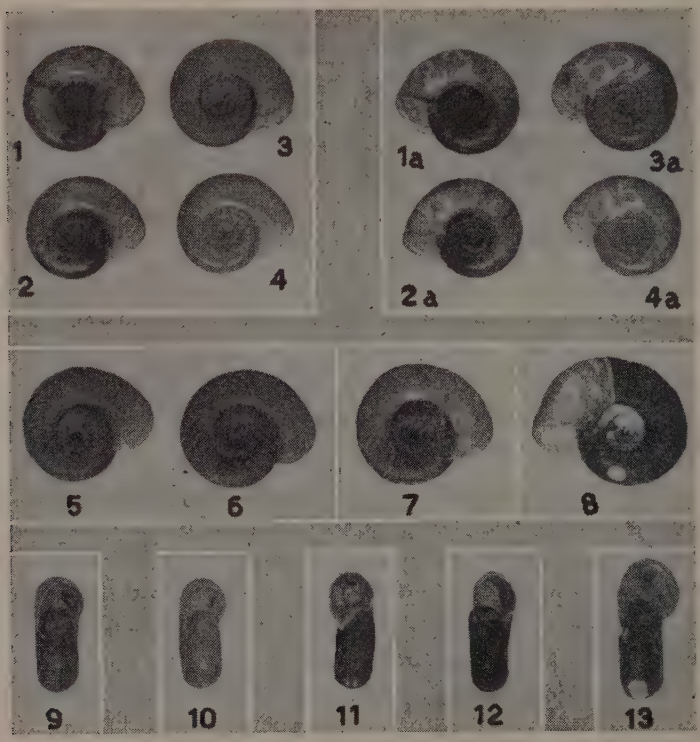
[1] Received for publication June 6, 1955.

MATERIAL AND METHODS

On December, 1954, we collected 302 specimens of *A. centimetralis,* the largest of which were 11 mm in shell diameter, fifty specimens with about 9 mm (8.6-9.2 mm) being selected for the present study. We examined comparatively fifty specimens of *A. glabratus* of equivalent size, from the same population studied in a previous paper (PARAENSE & DESLANDES, 1955a); unless otherwise stated, references to *glabratus* will apply to these small specimens. Serial histologic sections from two whole 9 mm-specimens of each species were also observed. The methods referred to in our previous papers on other species (1951a, b) were employed again in this work. All the measurements were made on camera lucida drawings.

SHELL

centimetralis, figs. 1, 1a, 2, 2a, 5, 6, 7, 8, 9, 11, 12, 13;
glabratus, figs. 3, 3a, 4, 4a, 10.



Figs. 1, 2 — *A. centimetralis;* figs. 3, 4 — *A. glabratus,* right side. Figs. 1a, 2a — *A. centimetralis;* figs. 3a, 4a — *A. glabratus,* left side. *A. centimetralis* — Fig. 5: Last whorl increasing more rapidly than usually; figs. 5, 6: oblique striation well apparent; fig. 7: inner whorls darker than outer ones; fig. 8: black-shelled field specimen showing natural pale color of the laboratory-grown portion. Figs. 9-13: Forms of shell aperture; 9: *A. centimetralis,* 10: *A. glabratus,* usual forms; 11-13: *A. centimetralis;* last whorl deflected to right in 11, to left in 12, aberrantly wide aperture in 13. [All figures enlarged twice].

The empty shell is yellowish or pale amber in color, shows a fine oblique striation, and has 3 3/4 to 5 whorls. In almost every specimen of our sample, however, the shell is covered with a black crust, the thickness of which decreases from the innermost to the outermost whorl, this indicating that it results from a deposition of environmental materials. The crust so firmly adheres to the shell as to become embedded between the whorls as the shell grows onward. In field collected animals which continue their growth in the laboratory the newly formed portion of the shell shows the natural pale color.

The whorls are smooth and evenly rounded on both sides. The right side is deeply umbilicated and more or less concave, or sometimes flattened beyond the umbilicus. The left side is more deeply concave than the right one. The whorls increase rapidly in diameter.

The shell aperture is more frequently a little higher than wide, but in many specimens it shows an approximately circular outline, and in still fewer cases it is a little wider than high. The aperture normally looks forward.

The shell width, taken at the aperture, was 2.8-3.7 (mean 3.23 ± 0.18)mm.

A comparison between the shell of *centimetralis* and *glabratus* shows that, with the exception of the latter being somewhat thinner on acount of their relative youth, they exhibit a remarkable resemblance. The shells of *glabratus* had 4-4 3/4 whorls and were 3.3-3.8 (mean 3.51 ± 0.11) mm wide at the aperture.

ANIMAL

The living animal shows numerous dark patches visible through the shell, corresponding to pigmented areas of the mantle. These patches may be more clearly seen in the mantle of the animal removed from the shell (fig. 15 *mp*). The pigmentation of the body is subject to individual variation, being generally moderate. The body length was 21-29 (mean 24.75 ± 1.85) mm.

The pigmentation of the mantle in *glabratus* of the population here concerned is almost uniformly black (fig. 16), on account of an early coalescence of the discrete spots existing in still younger animals. The body length was 20-29 (mean 24.44 ± 2.16) mm.

RENAL TUBE

centimetralis, fig. 15; *glabratus,* fig. 16.

The renal tube was 10.0-15.8 (mean 12.90 ± 1.20) mm long and 0.4-1.0 mm wide; the ureter was 1.0-1.8 mm long. The respiratory membrane covering ventrally the renal tube showed the smooth and unpigmented appearance described in *nigricans* (1955b). In *glabratus* this membrane is also smooth, but it shows a pigmented longitudinal line along which the longitudinal fold characterizing the species (renal ridge) will develop as the animal grows larger

(1955a). The renal tube of *glabratus* was 9.0-12.5 (mean 10.76 ± 0.89) mm long and 0.5-1.0 mm wide; the ureter was 0.6-1.8 mm long.

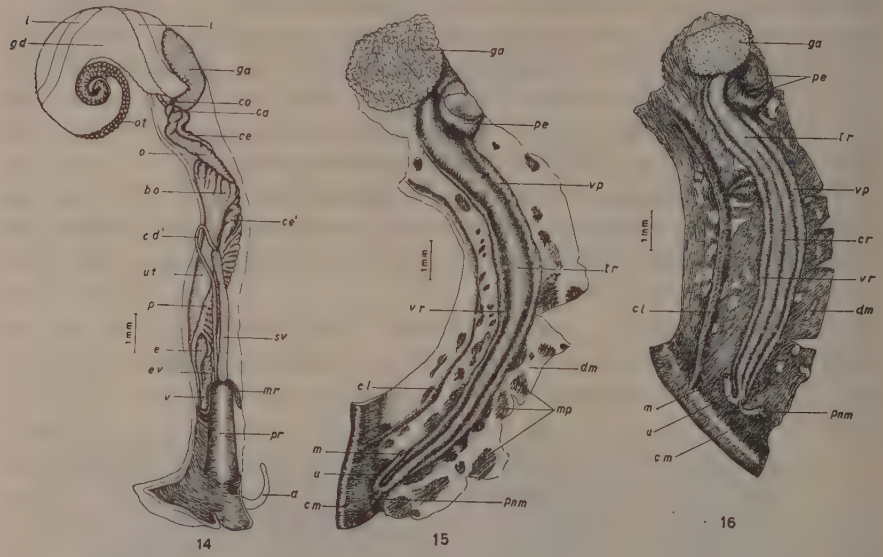


Fig. 14 — Body of *A. centimetralis* showing organ relations; fig. 15 — renal region of *A. centimetralis;* fig. 16 — renal region of *A. glabratus.* (a = antenna, bo = pouch of oviduct, ca = carrefour, cd' = distal segment of vas deferens, ce = sperm duct, ce' = wavy cephalic end of sperm duct, cl = longitudinal lateral ridge, cm = mantle collar, co = ovisperm duct, cr = pigmented line on future renal ridge, dm = roof of respiratory cavity, e = spermatheca, ev = vaginal corrugation, ga = albumen gland, gd = digestive gland, i = intestine, m = meatus of ureter, mp = pigmentary patches of mantle, mr = retractor muscles of preputium, o = oviduct, ot = ovotestis, p = prostate, pe = pericardium, pnm = pneumostome, pr = preputium, sv = vergic sac, tr = renal tube, u = ureter, ut = uterus, v = vagina, vp = pulmonary vein, vr = renal vein).

GENITALIA

centimetralis, fig. 17; *glabratus,* fig. 18.

The histological features resemble those of *glabratus* and *nigricans,* as previously described (1955a, b). Some of the anatomical characters are remarkably different from those described in the above mentioned species, giving support for the morphological characterization of *centimetralis.*

HERMAPHRODITE ORGANS

Ovotestis — Length 5.0-8.3 (mean 6.50 ± 2.77) mm; width at cephalic end 0.5-1.5 mm. The diverticula are short and stout, generally simple, less frequently bifurcate; at the cephalic portion of the organ, they are attached to the dorsolateral wall of the collecting canal (fig. 20, B, B').

In *glabratus* the ovotestis was 5.4-10.0 (mean 7.81 ± 1.12) mm long, and 0.7-1.2 mm wide at the cephalic end. The diverticula, beside being much more

numerous and predominantly trifurcate, are comparatively long and slender, showing a more distinctly fan-like appearance (fig. 19, A, A').

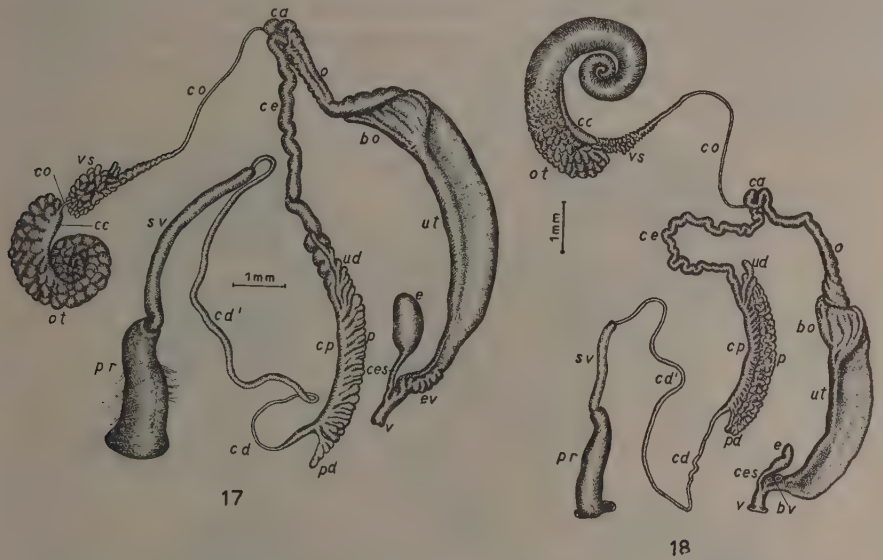


Fig. 17 — Genitalia of *A. centimetralis;* fig. 18 — genitalia of young *A. glabratus.* (bo = pouch of oviduct, bv = vaginal pouch, ca = carrefour, cc = collecting canal of ovotestis, cd = proximal segment of vas deferens, cd' = distal segment of vas deferens, ce = sperm duct, ces = spermathecal duct, co = ovisperm duct, cp = prostate duct, e = spermatheca, ev = vaginal corrugation, o = oviduct, ot = ovotestis, p = prostate, pd = foremost prostate diverticulum, pr = preputium, sv = vergic sac, ud = hindermost prostate diverticulum, ut = uterus, v = vagina, vs = seminal vesicle).

Ovisperm duct — Its length, including the non-unwound seminal vesicle, was 5.0-12.3 (mean 6.93 ± 1.29) mm; the width of the duct proper was about 0.10 mm. The greatest width of the seminal vesicle was 0.5-1.2 mm.

In *glabratus* the duct, plus seminal vesicle, was 5.2-11.5 (mean 6.43 ± 0.94) mm long; width of the duct about 0.10 mm, greatest width of the seminal vesicle 0.25-0.66 mm. The vesicle showed a comparatively rudimentary, juvenile appearance.

Carrefour — No special features were observed.

MALE ORGANS

Sperm duct — This organ was 5.0-11.2 (mean 6.94 ± 1.19) mm long and 0.16-0.33 mm wide (mode 0.25 mm, in 28 specimens). In *glabratus* it was 5.0 -10.8 (mean 6.86 ± 1.19) mm long and 0.12-0.20 mm wide (mode 0.16 mm, in 44 specimens). The small difference between the mean lengths is not significant statistically.

The sperm duct, adnate to the oviduct, follows a more sinuous course in *centimetralis* than in *glabratus;* whereas in the latter the cephalic portion of the

duct runs roughly straight, in *centimetralis* it shows a wavy appearance. As will be seen later, the oviduct is significantly longer in *glabratus*. Since the oviduct and sperm duct arise side by side from the carrefour, and have their cephalic ends at the same level, it should be expected that the shorter the oviduct will be, the more sinuous will appear the sperm duct as a result of the accomodation of its length to a relatively short pathway.

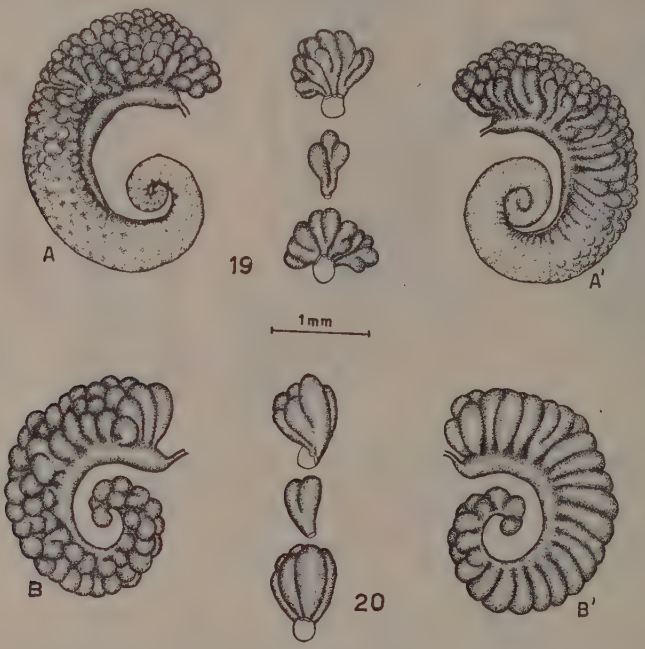


Fig. 19 — Ovotestis of young *A. glabratus*. A: right side; A': left side; middle: cross sections at cephalic portion showing fan-like appearance of diverticula. Fig. 20 — Ovotestis of *A. centimetralis*. B: right side; B': left side; middle: cross sections at cephalic portion showing chiefly simples diverticula.

The width of the sperm duct in *centimetralis* is larger than in *glabratus*, being comparable to that found in adult *glabratus* of 18 mm (1955a).

Prostate — The prostate duct, as in *glabratus* and *nigricans*, is merely the continuation of the sperm duct collecting the prostate diverticula. It measured 2.0-3.7 (mean 2.91 ± 0.35) mm. Its length in *glabratus* was 1.9-3.5 (mean 2.68 ± 0.32) mm, in this particular no appreciable difference existing between both samples.

The diverticula (fig. 21) are arranged in a single row of rather short and stout glandular tubes. Their number varied from 11 to 16 (mean 12.8 ± 1.3), no correlation being observed between those numbers and the length of the prostate duct. In *glabratus* they ranged from 15 to 30 (mean 22.5 ± 4.3). The diverticula are either simple or branched (bifurcate, trifurcate, arborescent). With the exception of the foremost and hindermost ones, that show some variation, and of aberrant units, all the others are consistently arborescent.

The hindermost diverticulum was arborescent in 40, trifurcate in 5, bifurcate in 5 cases; in *glabratus* it was arborescent in 6, trifurcate in 3, bifurcate in 19, simple in 22. The foremost diverticulum was arborescent in 40, trifurcate in 2, bifurcate in 4, simple in 4 cases; in *glabratus* it was arborescent in 46, bifurcate

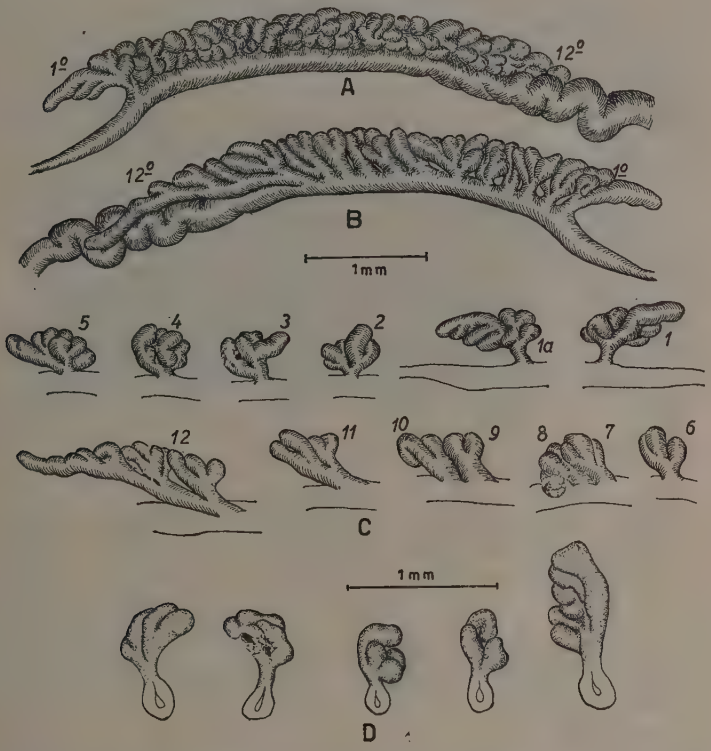


Fig. 21 — Prostate of *A. centimetralis*. A: side adherent to nidamental gland; B: side opposite to A; C: diverticula from 1st to 12th; D: diverticula as seen from interproximal sides.

in 4. Whereas all the intermediate diverticula were arborescent in *centimetralis*, some of them (the penultimate and antepenultimate ones) showed a varying character in *glabratus*: penultimate arborescent in 31, trifurcate in 2, bifurcate in 13, simple in 4 cases; antepenultimate arborescent in 47, bifurcate in 1, simple in 2. The stalks and branches of the diverticula may be clearly seen from the free lateral surface of the prostate (fig. 21 B). The diverticula tend to bend toward the side in contact with the nidamental gland (fig. 21 D), so that this side shows a confusing appearance due to crowding of the ends of the diverticula (fig. 21 A); in *glabratus* the diverticula bend to either side (fig. 22, nos. 9, 10, 17), so that ends of them may be seen from both lateral surfaces (fig. 22 A, B).

Vas deferens — This organ measured 7.5-14.5 (mean 11.59 ± 1.31) mm in length and about 0.20 mm in width. In *glabratus* it was 7.5-11.6 (mean 9.86 ± 1.19) mm long and about 0.10 mm wide.

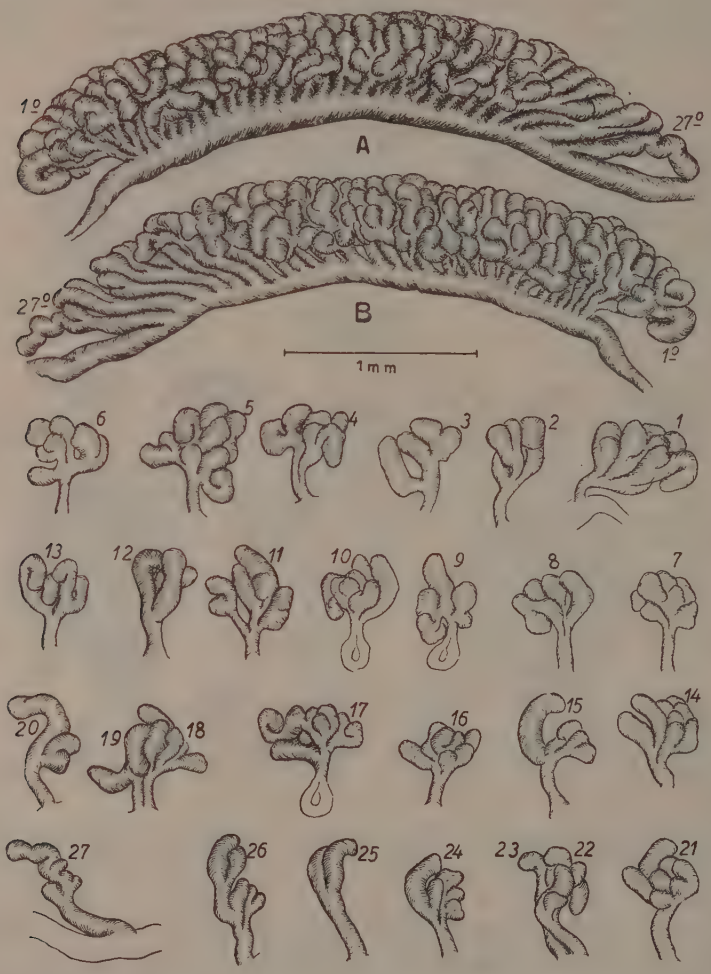


Fig. 22 — Prostate of young *A. glabratus*. A: side adherent to nidamental gland; B: side opposite to A; diverticula from 1st to 27th.

The ratio *vas deferens* : *vergic sac* ranged from 2.0 to 4.4 (mean ratio 3.17 ± 0.47); in *glabratus* 2.8-5.7 (mean 4.37 ± 0.63).

Penial complex — The *vergic sac* was 2.5-5.0 (mean 3.68 ± 0.50) mm long and 0.20-0.42 mm wide (mean 0.28, mode 0.25 with 27 items). In *glabratus* it was 1.6-2.8 (mean 2.28 ± 0.29) mm long and 0.12-0.20 mm wide (mean 0.16, mode 0.16 with 43 items). The length values were highly significantly greater in *centimetralis*. Their distribution, with the only exceptions of two

lower overlapping items (2.5 and 2.6 mm), ranged above that of *glabratus*. The length of the vergic sac in the present sample of *centimetralis* is practically the same as in adult *glabratus* with 18 mm in shell diameter. As to the width of the vergic sac, its lowest value in *centimetralis* corresponded to the highest value in *glabratus* (one item of 0.20 mm for each sample). The vergic sac of *centimetralis* is significantly wider than that of even adult *glabratus* with 18 mm, in which it was 0.19 mm in mean width.

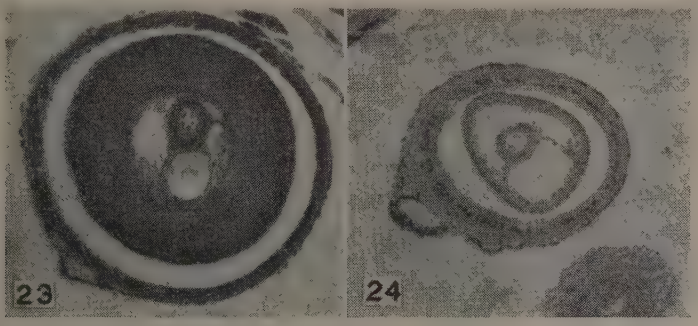


Fig. 23 — Verge of *A. centimetralis*, within vergic sac. Fig. 24 — Verge of adult *A. glabratus* (18 mm in shell diameter), within vergic sac. Both figs. enlarged 200 ×.

The ratio *vergic sac* : *preputium* varied from 0.9 to 2.3 (mean ratio 1.35 ± 0.25). In *glabratus* it was 0.7-1.4 (mean 1.05 ± 0.18).

The *verge* showed the same length as the vergic sac enclosing it, its width being a little smaller than that of the sac. So, the verge of *centimetralis* is much wider than that of *glabratus*. A conspicuous histological character distinguishes the verge of *centimetralis* from that of *glabratus* (and *nigricans*). In the latter "the verge is covered with a visceral squamous epithelium that loops back at the base of the organ to become continuous with the parietal epithelium lining the vergic sac. The sperm canal is lined by a low epithelium continuous with the lining of the vas deferens. The lacunary vergic tissue fills the space between the two epithelial layers" (Paraense & Deslandes 1955a). In addition to this description we state now that a thin circular muscular layer intervenes between the visceral epithelium and the lacunary tissue proper (fig. 24). In *centimetralis* the muscular coat is remarkably thicker and arranged in three layers, of which the inner and outer ones are longitudinal, and the middle one circular (fig. 23). Toward the base of the verge the outer layer gradually attenuates, so that it ultimately disappears, thus becoming the muscular coat reduced to the inner and middle layers.

The *preputium* was 1.8-3.4 (mean 2.76 ± 0.29) mm long and 0.58-1.00 mm wide (mean 0.78, mode 0.80 with 21 items). In *glabratus* it was 1.6-2.9 (mean 2.22 ± 0.36) mm long and 0.33-0.66 mm wide (mean 0.50, mode 0.50 with 15 items).

FEMALE ORGANS

Albumen gland — With the exception of its being relatively larger in *centimetralis,* no special features were observed.

Oviduct — Its dimensions were 2.9-5.4 (mean 4.13 ± 0.56) mm in length and 0.25-0.58 mm in width; the region of the pouch was 0.9-1.6 mm wide. In *glabratus* 3.3-5.8 (mean 4.56 ± 0.57) mm in length and 0.20-0.66 mm in width; the pouch was 0.33-1.66 mm wide. The oviduct in *centimetralis* was significantly shorter than in *glabratus,* the difference between the mean lengths being 3.8 times its standard error.

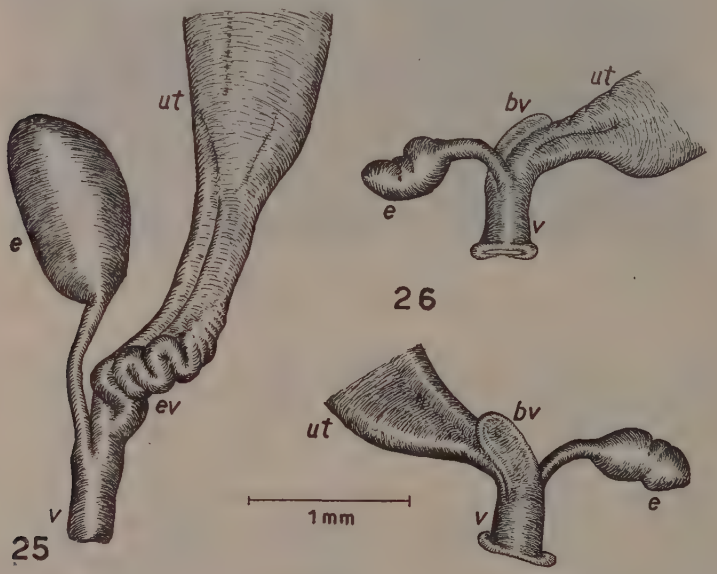


Fig. 25 — Cephalic end of female genital organs of *A. centimetralis.* Fig. 26 — Idem of young *A. glabratus,* from two opposite sides.

Uterus — 5.0-8.3 (mean 6.03 ± 0.69) mm long, 0.5-1.4 mm wide; in *glabratus,* 3.3-5.8 (mean 4.69 ± 0.56) mm long, 0.5-1.4 mm wide. The general conformation is the same as in the previously studied species, *glabratus* and *nigricans,* the limit between the nidamental gland and the uterus proper being also indistinct.

Vagina — 1.8-2.9 (mean 2.43 ± 0.24) mm long, 0.2-0.5 mm wide. In *glabratus,* 0.8-1.5 (mean 1.09 ± 0.18) mm long, 0.2-0.5 mm wide.

The external appearance of the organ is remarkably different from that observed in *glabratus* and *nigricans.* In these species the caudal portion shows a lateral swelling (vaginal pouch), the cephalic limit of which is situated at the level of the attachment of the spermathecal duct (fig. 26 *bv*). In *centimetralis* the homologous portion of the vaginal wall constantly showed a corrugated external surface, instead of a smooth-walled pouch (fig. 25 *ev);* moreover, its

vagina was found to be highly significantly longer than those of 18 mm — *glabratus* and *nigricans* (mean lengths 2.06 ± 0.15 and 1.70 ± 0.22 mm respectively), the differences between the means in each case being respectively 9 and 16 times their standard errors.

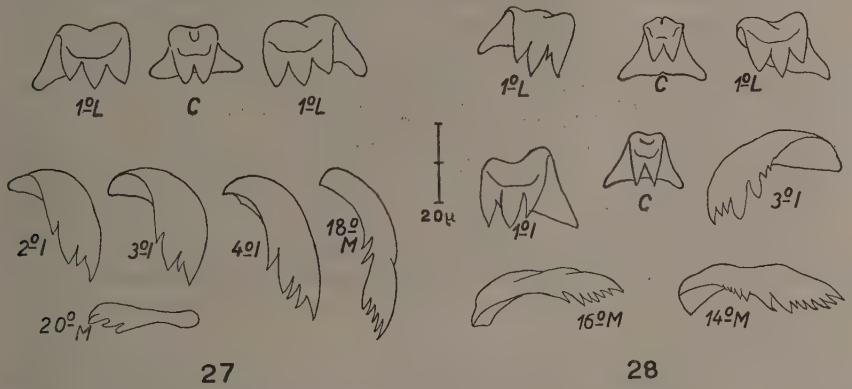
Spermatheca — The body of the spermatheca was 0.9-2.0 (mean 1.33 ± 0.24) mm long and 0.42-1.00 (mean 0.63 ± 0.12) mm wide; the spermathecal duct was 0.58-1.20 (mean 0.90 ± 0.12) mm long and about 0.10 mm wide.

In *glabratus* the body was 0.50-1.16 (mean 0.79 ± 0.18) mm long and 0.25-0.66 (mean 0.45 ± 0.13) mm wide; the duct was 0.4-1.0 (mean 0.62 ± 0.12) mm long and about 0.10 mm wide.

RADULA

centimetralis, fig. 27; *glabratus,* fig. 28.

The number of teeth rows varied from 105 to 134 (mean 118.9 ± 7.7). The radula formula ranged from 21-1-21 to 30-1-30 (mean 24.9 ± 2.2). In *glabratus* there were 105-135 (mean 119.0 ± 6.6) rows, and the radula formula varied from 20-1-20 to 25-1-25 (mean 22.3 ± 1.1).



27 28

Fig. 27 — Radula teeth of *A. centimetralis.* Fig. 28 — Radula teeth of young *A. glabratus.* C, central; L, lateral; I, intermediate; M, marginal.

In both species, the cusps of the central tooth varied from shorter to longer than the base of attachment, in the latter case extending below the lower margin of the base; the relative dimensions of the tooth were variable, its height surpassing its width or vice-versa, these two dimensions being sometimes equivalent. There were 6 to 13 laterals in *centimetralis,* and 5 to 7 in *glabratus.* The intermediates ranged from 2 to 4 in both species; we considered as intermediate those teeth showing one or two interstitial cusps between the entocone and mesocone. The marginals were 9 to 17 in *centimetralis,* and 11 to 16 in *glabratus.*

The teeth showed no appreciable interspecific morphological differences.

DISCUSSION

In the present paper the morphological characters allowing a distinction between *A. centimetralis* and the two previously studied species *A. glabratus* and *A. nigricans* will be considered exclusively. In a further paper the biological characterization of the species under investigation will be presented.

Separation from young *nigricans* may be made at once by the shell characters, at least those exhibited by populations with typical shell pattern as described in our mentioned paper (1955b); the latter refers to specimens of 18 mm in diameter, but comparison should be made on qualitative basis. On the other hand, separation from young *glabratus* on conchological basis exclusively requires a certain degree of experience in recognizing the "adult" character of the small shell of *centimetralis;* if dealing with a reasonably large sample, the observer will have additional information supplied by the greatest shell diameter.

In living snails the dark patches of the mantle, visible through the shell, generally distinguishes an adult *centimetralis* from an equivalently sized *glabratus*. The mantle pigmentation, however, should be cautiously considered, as it is subject to group variation in *glabratus*. In ordinary populations of the latter the pigmentary patches occur in very young specimens (up to about 5 mm), the pigmentation soon spreading over the whole mantle as the snail grows larger. In some populations exhibiting a lighter body pigmentation, as those occurring in the districts of Medina, Derribadinha and Januaria (State of Minas Gerais), the coalescence of the pigmentary patches in the mantle becomes delayed, so that a spotted appearance may be clearly observed in specimens with 10 mm in diameter. Recently we made a particularly remarkable observation on this subject in a population of *glabratus* from Derribadinha, which after the rainy season became reduced to nearly only young specimens showing the mentioned spotted appearance; in this case we remained in doubt as to the identity of our sample until the examination of the internal organs was carried out.

The renal region in *centimetralis* and *glabratus* shows the differences referred to in the description of the renal tube. According to our present experience in Brazilian planorbids, a renal ridge or the pigmented line preceding it occurs in a single species, *A. glabratus*. Thus, the character of the renal region is decidedly important for separation of *glabratus* from all the other species, but is inoperative among the latter.

As to the ovotestis, its diverticula differ remarkably in number and appearance from those of *glabratus* and *nigricans*.

The prostate shows some peculiar characters which will prove useful in systematic work. The mean number of its diverticula was highly significantly less than in *glabratus* and *nigricans*. This character, however, cannot be adequately appreciated in routine work, requiring relatively large sampling for statistical analysis, since its distribution overlaps the least values recorded in

the two other species. The appearance of the diverticula and the degree of complexity of the caudal ones must be considered as convenient taxonomic characters. As may be seen in the above description of the prostate, the general branching pattern of the diverticula agrees with that described in *glabratus* and *nigricans*. In *centimetralis*, however, the branches are rather short and stout, so that at a cursory examination from the free surface of the prostate the diverticula seem as if they were divided into only primary branches. In addition, there is a noticeable tendency to an increased branching of the caudal diverticula as compared with *glabratus* and *nigricans*. In the first examined sample of *glabratus* (1955a), the hindermost diverticulum was simple in 29, bifurcate in 16, trifurcate in 1, arborescent in 4 cases; in the present sample of that species it was simple in 22, bifurcate in 19, trifurcate in 3, arborescent in 6 cases. A similar tendency was recorded in *nigricans* (1955b): simple in 8, bifurcate in 20, trifurcate in 2, arborescent in 20. As we have seen, there was no simple hindermost diverticulum in the present sample of *centimetralis*, whereas there was 5 bifurcate, 5 trifurcate, and 40 arborescent.

The ratio *vas deferens* : *vergic sac* showed no usability as taxonomic character in the three species here involved, since its distribution widely overlapped in them. To a certain degree, this statement applies to the ratio *vergic sac* : *preputium*, in spite of its being more frequently mentioned in systematic work. As regards *A. glabratus*, for example, it has been stated that the vergic sac is about half as long as the preputium (Pilsbry, 1934), slightly shorter (Baker, 1945), equal to or a little longer (Coutinho, 1949), generally longer (Barbosa & Dobbin, 1951) than the preputium. The above contradiction is only apparent, since it actually expresses the expected variation of the character. There should be primarily an intrinsic variability in the length of both organs, which would be measurable if an ideal state of relaxation could be obtained. In addition to such intrinsic variability, another cause of variation is represented by the state of contraction of either organ at the moment of death. The contradiction among the mentioned statements results from the insufficient size of the samples examined. As a matter of fact, the above authors do not refer to the numbers of specimens used in their work. If a reasonably large sample is dealt with (Pinto & Deslandes, 1953, Paraense & Deslandes, 1955a, and present paper), the tendency of the ratio values to crow around 1 is observed, signifying that in *glabratus* both vergic sac and preputium tend to be equally long, the values less and greater than 1 being expected to be equivalently frequent. In *centimetralis*, the vergic sac shows a noticeable tendency to be longer than the preputium, the mean ratio being 1.35 ± 0.25. From the statistical viewpoint, this value is highly significantly greater than those recorded in 18 mm-*glabratus* and *nigricans*, and 9 mm-*glabratus*, the differences between it and those of the latter being respectively 10, 13 and 7 times their standard errors. In spite of this character showing less overlap than that observed in the ratio *vas deferens* : *vergic sac*, the very possibility of such

overlap makes it unusable in routine work, unless associated with the other distinctive characters pointed out in the present paper. Among the latter we make mention to the relatively great length of the penial complex as compared with the whole body length (fig. 14). In addition to its relatively greater length, the vergic sac looks wider than in the previously studied species. Whereas in the latter its width did not exceed 0.20 mm, in *centimetralis* this figure corresponds to the least record observed in a single specimen.

As concerns the female organs, the ratio *uterus* : *oviduct,* which was not mentioned in our previous papers, seems to deserve some consideration. Its values ranged from 0.7 to 1.0 (mean 0.82 ± 0.07) in 18 mm-*nigricans,* 0.8 to 1.3 (mean 1.04 ± 0.12) in 18 mm-*glabratus,* 0.8 to 1.3 (mean 1.03 ± 0.10) in 9 mm-*glabratus,* and 1.1 to 2.3 (mean 1.48 ± 0.24) in *centimetralis.* The figures recorded for both samples of *glabratus* are equivalent, the difference between their means having no significance (0.4 times the standard error). On the other hand, the differences between the means of *nigricans* and *glabratus* (pooled sample), as well as of the latter and *centimetralis,* were highly significant (14 and 12 times the s.e., respectively).

The corrugation observed on the wall of the vagina is a peculiar character of *centimetralis,* since as yet we have found it in no individual either of the previously studied, or of any other supposedly different species.

In conclusion we may say that, putting aside the size of the specimens under consideration, we should apply the following criteria for the morphological distinction between the three species till now studied by us. By shell inspection, we should separate as *nigricans* the wide-shelled, carinate specimens; by examination of the renal region, the specimens showing renal ridge (or the pigmented line in small specimens) would be separated as *glabratus;* a further distinction between *centimetralis* and *nigricans* would be made by the presence of a corrugated vaginal wall in the former and of a smooth vaginal pouch in the latter. In our opinion, a sound characterization of *centimetralis* can be made on the basis of the features described in its ovotestis, prostate (including wavy cephalic end of sperm duct), vergic sac, oviduct (shorter than uterus), and vagina, considered conjointly.

SUMMARY

A description of the shell, renal region, genital organs and radula of 50 specimens of *Australorbis centimetralis* from a population occurring in Governador Valadares (State of Minas Gerais) is presented in this paper. A comparison is made between this sample and another of equivalently sized *A. glabratus.* The characters which permit separating *centimetralis* from the previously studied species *glabratus* and *nigricans* are considered in the following order: Shell carinate and relatively wide in *nigricans,* smooth, without carinae, relatively narrow in *glabratus* and *centimetralis;* renal region with ridge (or

pigmented line in small specimens) in *glabratus,* smooth and unpigmented in the other two; corrugated vaginal wall in *centimetralis,* smooth vaginal pouch in the others. In addition to the mentioned alternative characters, recognition of *centimetralis* can be made by the following attributes, which must be considered conjointly: ovotestis diverticula short, stout, generally simple, less frequently bifurcate, less numerous than in the two other species; sperm duct showing a wavy appearance in the immediate vicinity of the prostate; prostate with short, stout diverticula, of which the hindermost one is nearly always arborescent, the penultimate and antepenultimate being normally arborescent; vergic sac rather wide, generally longer than the preputium; penial complex relatively longer than in the other species, as compared with the body length; uterus generally longer than the oviduct.

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CARÊNCIA DE COBALTO EM BOVINOS NO ESTADO DE SÃO PAULO [1]

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A carência de cobalto como doença dos animais domésticos tem sido estudada em certas regiões de vários países, principalmente Austrália, Nova Zelândia e Estados Unidos. Uma das características interessantes dêste tipo de carência está no fato de ser exclusiva dos ruminantes.

A importância do cobalto como elemento indispensável à alimentação dêsses animais era desconhecida até a pouco tempo, e só a partir de 1935, na Austrália, é que o assunto foi esclarecido por meio de um conjunto de dados de observações clínicas e de experimentação. Dêsses estudos constatou-se a grande eficiência que tem o cobalto como elemento mineral essencial à nutrição dos ruminantes. Sua importância está no fato de ser um elemento constituinte da molécula da vitamina B_{12}, fator anti-anêmico e de crescimento, que é sintetisada no rumen por microorganismos.

No Brasil o problema não foi até agora objeto de qualquer trabalho de observação ou de experimentação. Julgamos assim indicado procurar esclarecer se o quadro clínico descrito para esta carência naquelas regiões, aqui se verificava e, em seguida, se análises complementares determinariam a existência no país dêste tipo de carência mineral.

Sob o ponto de vista clínico a carência de cobalto apresenta um quadro de sintomas de hipoalimentação, anorexia, anemia, caquexia, alotriofagia, debilidade, andar cambaleante, diarréia intensa muito freqüente, ou, às vêzes, constipação, diminuição da produção de leite, pêlo crescido, áspero e arrepiado; as fêmeas prenhes geralmente abortam e a natalidade no rebanho é diminuida, etc...

Por inquérito clínico levado a efeito no interior do Estado de São Paulo, pareceu-nos que a êsse quadro deviam corresponder os casos de doença freqüentes em bovinos no nosso meio, e conhecidos vulgarmente pelos nomes de "peste de secar", "mal do colete", "sablose", "mal de areia", "mal das cabeceiras", e outros nomes regionais. As verminoses e a ingestão de areia anteriormente

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apontadas como causas desta doença não nos pareceram explicar de modo completo o desenvolvimento dêste síndrome; e, além disso, êstes dois fatôres foram responsabilisados em época em que nada se conhecia ainda sôbre a importância dos microelementos na alimentação, e especialmente do cobalto para os ruminantes.

No presente trabalho procuramos fornecer alguns dados preliminares para a comprovação da existência da carência do cobalto em bovinos no nosso meio, em diversas regiões do Estado. Em algumas regiões, como na Flórida, Estados Unidos, tem sido assinalada uma dupla carência de cobalto e cobre. No nosso meio existe apenas a de cobalto, como mostram as análises incluidas na tabela 1.

Na Nova Zelandia, na Austrália, nos Estados Unidos e em Kenia, o quadro clínico descrito pelos diversos autores que estudaram o assunto se superpunha em linhas gerais, ao que aqui se verifica e havia pois, interêsse em examinar na mesma direção a doença aqui constatada.

TABELA 1

Teor de cobalto e cobre encontrado no fígado de bovinos sadios e doentes. Cobalto e cobre expressos em p.p.m. sôbre a matéria seca.*

ANIMAIS SADIOS			ANIMAIS DOENTES		
N.º	Cobalto	Cobre	N.º	Cobalto	Cobre
1	.113	139.2	1	.038	508.2
2	.113	241.6	2	.056	358.0
3	.120	266.5	3	.060	198.9
4	.126	118.2	4	.063	163.3
5	.127	295.0	5	.065	180.0
6	.150	420.3	6	.069	144.5
7	.172	373.2	7	.075	223.0
8	.174	148.0	8	.079	240.0
9	.193	157.9	9	.087	105.8
10	.200	304.4	10	.090	196.0
11	.267	60.0	11	.090	86.1
12	.268	73.2	12	.100	315.5
13	.289	152.1			
14	.314	97.8			
15	.328	52.6			
Médias	.197±.020	191.5±30.2	Médias	.073±.005	226.6 ±34.1**

* — De acôrdo com os diversos autores que estudaram o assunto, os índices para cobalto e cobre no fígado dos bovinos normais e com carência dêsses microelementos são os seguintes: (valores em partes por milhão sôbre a matéria sêca)

Cobalto . normal > .100 — Carência < .080
Cobre . normal > 100. — Carência < 50.

** — O número precedido de ± é o êrro padrão da média.

A doença, de quadro clínico semelhante ao que é descrito naqueles países, pode ser encontrada com freqüência em várias regiões do Estado, na zona da Noroéste, na Sorocabana, na Paulista e no Vale do Paraíba. Ela ocorre com maior freqüência em determinada época do ano, geralmente de dezembro a maio, quando os bovinos são atingidos em maior número, com sintomas mais graves e levando-os mesmo à morte.

Nossos estudos indicam que o aparecimento da doença em nosso meio depende da deficiência de cobalto nas forragens consumidas pelo gado.

Foram feitas análises microquímicas quantitativas do teor em cobalto no fígado de bovinos sadios e, comparativamente, análises idênticas de fígado de animais doentes. Os índices obtidos nos dois casos mostram diferenças acentuadas no teor em cobalto. Essas diferenças correspondem em linhas gerais às que têm sido assinaladas pelos autores que examinaram o problema em outros países. Foram igualmente feitas análises do teor em cobalto nas forragens das invernadas que alimentavam o gado doente, com índices abaixo do teor mínimo necessário, e de invernadas onde a doença não ocorria.

A técnica de trabalho seguida nessas análises de fígado de animais sadios e doentes consistia em colher cêrca de 100 gramas de fígado em frascos de 300 ml de capacidade, que eram previamente lavados com ácido, a fim de evitar possíveis impurezas, utilisando-se como conservador o álcool etílico redestilado em aparêlho de vidro. O fígado era cortado em pequenos fragmentos e deshidratado em forno a 100°C., durante 24 horas. A determinação do cobalto foi realisada pela técnica do australiano MARSTON, que consiste no desenvolvimento do complexo nitroso-R-sal-cobalto. Entretanto, em vez do método colorimétrico usado por êste autor, nossas dosagens fram realizadas no espectrofotômetro Coleman Junior, Modelo 6-A, em cubas de 12 X 75 mm; e a curva padrão construida com pontos de 0.5, 1.0, 1.5 e 2.0 microgramas de cobalto em 10 ml. As leituras foram feitas em onda de 420 milimicrons e lidas em densidade ótica.

A tabela 1 fornece indicações sôbre o número de análises realisadas em fígado de animais normais e doentes, assim como indica o teor em cobalto nos dois casos. Pode-se verificar claramente a deficiência nos casos de animais doentes.

A quantidade de cobalto dosada em fígado de bovinos sadios é superior a 0.100 p.p.m. sôbre a matéria seca, ao passo que em bovinos doentes o teor de cobalto no fígado fornece índices muito inferiores.

A administração de cobalto, adicionado ao sal comum, permite o restabelecimento completo dos animais em adiantado estado de doença.

SUMÁRIO

No Brasil o estudo da carência de cobalto como fator de doença e de mortalidade de bovinos não foi até agora objeto de qualquer trabalho de observação clínica e de experimentação. Procuramos neste trabalho assinalar a presença em extensas áreas do interior do Estado de São Paulo, do quadro clínico descrito em bovinos nesse tipo de carência em outras regiões.

O síndrome conhecido no nosso interior sob os nomes de peste de secar, mal de colete, sablose, mal de areia, etc., caracterisado por caquexia, letargia, anorexia e anemia é uma carência de cobalto, idêntica ao marasmo enzoótico da Austrália e o "bush-sickness" da Nova Zelândia.

Foram feitas análises microquímicas quantitativas do teor em cobalto no fígado de bovinos doentes e normais. Os índices obtidos revelam diferenças

nítidas e acentuadas nos dois casos. Estas diferenças correspondem às que são assinaladas pelos autores que estudaram o problema em outras regiões. Nos animais normais o teor em cobalto em 15 análises realisadas oscila entre .113 e .328 p.p.m. sôbre a matéria seca, sendo a média de .197 ± .020. Nos animais doentes, em 12 casos examinados, o teor em cobalto oscila entre .038 e .100 p.p.m., sendo a média de .073 ± .005. Em uma única dosagem tivemos o índice de cobalto igual a .100 p.p.m. sôbre a matéria seca.

Estudos mais completos sôbre o assunto serão brevemente publicados.

SUMMARY

Cobalt deficiency as a cause of disease and mortality in cattle was not previously studied in Brazil either clinical or experimentally. The present paper reports the occurrence of the clinical symptoms of this mineral deficiency affecting cattle in large areas of the State of São Paulo. The disease, which in these rural areas receives several denominations related particularly to the observed ematiation and silt-covered rumina, is identical to the "enzootic marasmus" of Australia and the "bush-sickness' occuring in New Zealand. The main symptoms are cachexia, lethargy, inappetency and anemia.

Microchemical cobalt quantitative determinations were performed in the livers of normal and diseased cattle. Marked differences, corresponding to those observed in other countries, were found between the two groups of animals. In 15 normal animals, the cobalt content of liver varied between .113 and .328 p.p.m. of dry matter; in 12 sick animals, the values ranged between .038 and .100 p.p.m., the latter figure being observed in just one case. The arithmethic means and corresponding standard errors of the forementioned values were .197 ± .020 and .073 ± .005 for the groups of normal and diseased animals, respectively.

A more complete publication on these studies is forthcoming.

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AUSTRALIAN SPECIES OF THE GENUS "BLAESOXIPHA" LOEW, 1861 (Diptera, Sarcophagidae) [1]

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(With 10 text-figures)

There are two species belonging to the genus *Blaesoxipha* Loew among the specimens I have received from the Department of Entomology, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia. I am very gratefull to Dr. A. J. NICHOLSON for the opportunity to study this valuable collection.

HARDY (1943) includes *Locustivora pachytyli* (Skuse) in the genus *Blaesoxipha* Loew and describes *B. gemina* Hardy. I agree with HARDY because an accurate comparison between the male genitalia of *pachytyli* Skuse and *B. laticornis* Meigen, the type species of *Blaesoxipha,* proves the affinities of the two species. I dont found *B. gemina* Hardy but a very different species among the material from Australia and I sent to Dr. G. H. HARDY the figures I am publishing in this paper. I received from this colleague a letter in which he states that the species is unknown to him. I have appreciated very much the kindness of this valuable information from the best specialist in Australian Sarcophagidae.

Blaesoxipha pachytyli (Skuse) is somewhat related, by the shape of the *forcipes superiores* and *f. inferiores* to some species considered by ROHDENDORF (1937) as *Blaesoxipha (s. str.).* The *forcipes superiores* of *B. hardyi* sp.n. is similar to those of *B. kastneri* Baranoff from Sumatra, but the spines of the *forcipes* are limited to the median posterior part.

Blaesoxipha pachytyli (Skuse, 1891) Hardy, 1936
(Figs. 1-6)

Masicera pachytyli Skuse, 1891 : 251
Sarcophaga pachytyli Coquillet, 1892 : 22

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Sarcophaga pachytyli Froggatt, 1905 : 20
Sarcophaga pachytyli Froggatt, 1907 : 315
Sarcophaga pachytyli Johnston & Tiegs, 1922 : 175
Locustivora pachytyli Johnston & Tiegs, 1922 : 187-190
Blaesoxipha pachytyli Hardy, 1936 : 95-96
Opsophyto pachytyli Townsend, 1938 : 88
Blaesoxipha pachytyli Hardy, 1943 : 20, fig. 1

Male. Length 6 to 8 mm.

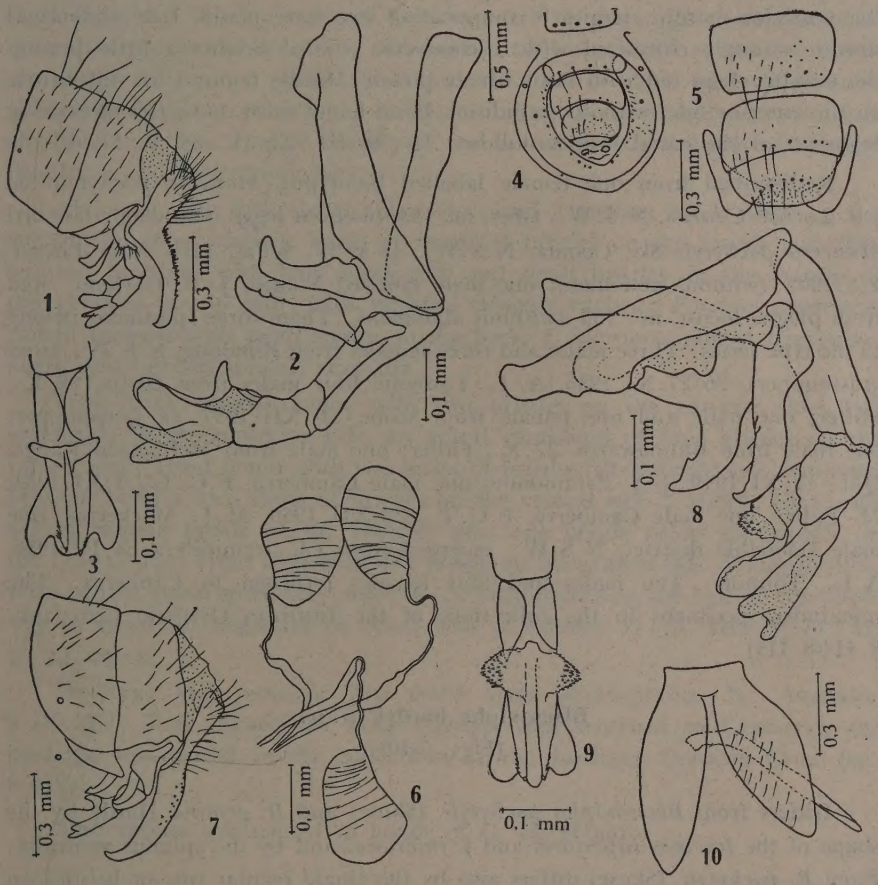
Head silvery. Front about 0,29 of head. Frontalia gray. Ocellar bristles present, outer verticals small. Parafacialia with two or three rows of hairs, sometimes only one irregular row, below near the eyes, the lower hairs are bristle-like. There are 9 to 11 frontal bristles, descending to the middle of the second antennal joint, two or three bristles, placed below the antennal level are widely diverging. Third antennal joint gray, second reddish-gray, sometimes red, with 0,44 of the length of the third. Antennae reaching 0,8 of the way to the vibrissal level. Vibrissae well above oral margin. Palpi brown or reddish-brown, sometimes red. Parafacialia 0,4 of the distance between the vibrissae. Arista short plumose on the basal half or less. Back of head with two rows of black hairs besides the postocular cilia, the remainder hairs are yellowish-white. Genae with black hairs only.

Thorax with yellowish gray pollen. Three postsutural supralar bristles, two strong presutural supralars; three to four postsutural intralars, having sometimes one or two small bristles between the postsutural intralars and the postsutural supralars; three presutural intralars; three strong postsutural dorsocentrals, sometimes one additional bristle in front; three presutural dorsocentrals; three to four strong presutural acrostichals; four strong postsutural acrostichals, including the prescutellar; two marginal scutellars, apical and preapical scutellars present; sternopleural bristles: 2 : 1; six to eight hypopleurals. Propleura and prosternum bare.

Abdomen gray with a median black stripe on the second and third tergite and changeable lateral stripes; third tergite with a pair of median bristles, fourth and fifth tergites with marginal rows. Sternites one to four with long black lateral pile and small bristles on the posterior end of the sternites two to four. Fifth sternite strongly excised with small hairs. *Forcipes superiores* with free part bent back and with numerous spines; *forcipes inferiores* small, excissed at tip, distal lobe slender. Penis with long theca, bilobed small *ventralia,* large *ductus ejaculatorius* and trilobed *preputium.*

Legs black. Middle femur with one or two median bristles on the anterior side; two or three preapical bristles on the posterior side; a small ctenideum composed of three or four strong bristles on the ventral side. Hind femur with two series of bristles on the anterior side, the inferior one composed of small bristles; two series of bristles on the ventral side, having one long bristle near the apex. Middle tibia with one very long median bristle on the anterior

side; one bristle below the middle on the ventral side. Hind tibia with two bristles on the ventral side. Wings hyaline, veins yellow, costal spine small, R_{4+5} hairy two-thirds way to cross vein. Segments of costal vein as follows: II : 64, III : 24, IV : 103, V : 32, VI : 4.



Blaesoxipha pachytyli (Skuse, 1891) — Fig. 1: Male genitalia, side view; fig. 2: penis and claspers; fig. 3: apex of penis, ventral view; fig. 4: female genitalia; fig. 5: female genitalia sternites; fig. 6: spermathecae. *Blaesoxipha hardyi* sp. n., male — Fig. 7: Genitalia, side view; fig. 8: penis and claspers; fig. 9: apex of penis, ventral view; fig. 10: fifth abdominal sternite.

Female. Length 4 to 6 mm.

Head silvery, front about 0,27 of head. Frontalia reddish, ocellar bristles strong, outer verticals about two-thirds the length of the inner verticals. Parafacialia with a row of hairs below near the eye, the lower three or four are bristle like. There are 8 to 10 frontal bristles. Antennae more reddish than in male, second joint 0,56 of third. Palpi red. Thorax gray, the stripes fainter

than in male. Two or three postsutural supralar bristles; anterior presutural supralar small; two or three postsutural acrostichals, only the prescutellar bristle strong; apical scutellar bristle absent. Sternopleurals: 1 : 1. Hypopleurals 4 to 6. Abdomen gray, stripes very faint. Abdominal sternites with a few small hairs and bristles on the posterior margin; tergite 6+7 entire, with bristles on the posterior margin; tergite 8 composed of two bare plates. Last abdominal sternite strongly chitinized with a transverse row of bristles a little beyond the middle. Legs red with faint silvery pollen. Middle femur with one bristle on the anterior side, without ctenidium. Hind femur with one series of bristles. Segments of the costal vein as follows: II : 59, III : 22, IV : 88, V : 31, VI : 5.

Redescribed from one female labelled holotypus, *Masicera pachytyli* Sk., par. Locust, Corowa, N.S.W., Grey, nr. 153 (without legs); one female labelled *Masicera pachytyli* Sk., Cooma, N.S.W., D & W. Sellar, bred from Locust, 4.3.1902 (without abdomen); one male labelled Wagga, J. B. Garland, bred from plague locust, nr. 153 (without abdomen). These three specimens belong to the type series. Three males and three females from Binalong, N.S.W., from grasshoppers, 26/27.XI.1935, A. L. Tonnoir; four males from Badja, 28.X., Fuller; one male and one female from Scone, 20.XII.1937, ex grasshopper; one male from Bunnawarra, 27.X., Fuller; one male from McPherson Range, Qld., 12.XI.1949, J. S. Paramonov; one male Camberra, F.C.T., 3.XI.1934, M. Fuller, one male Camberra, F.C.T., 22.XII.1930, M.J. Mackerras; one male Coonable destrict, N.S.W., emerged from Ch. terminifera 28.IV.1936, A.L. Tonnoir. Ten males and four females returned to Canberra. The remainder specimens in the collections of the Instituto Oswaldo Cruz (nr. 8.414-8.415).

Blaesoxipha hardyi sp. n.
(Figs. 7-10)

Differs from *Blaesoxipha pachytyli* (Skuse) and *B. gemina* Hardy by the shape of the *forcipes superiores* and *f. inferiores* and by the spinous ventralia. From *B. pachytyli* (Skuse) differs also by the single regular row of bristles on parafacialia and by the absence of median marginal bristles on the third abdominal tergite.

Male. Length 9 mm.

Head silvery. Front about 0,17 of head. Frontalia black. Ocellar bristles present, outer vertical bristles not differentiated. Parafacialia with a single regular row of bristles below, near the eyes. There are 9 to 12 frontal bristles descending to the middle of the second antennal joint, two bristles, placed below the antennal level, are diverging. Third antennal joint gray, first and second reddish, the later about 0,6 of the length of third. Antennae reaching

0,8 of the way to vibrissal level which are well above oral margin. Palpi red. Parafacialia with 0,5 of the distance between the vibrissae. Arista plumose on the basal half. Back of head with one or two rows of black hairs besides the postocular cilia, the remainder hairs are white. Genae with black hairs only.

Thorax yellowish gray with three postsutural supralar bristles, two presutural supralars; three postsutural intralars, two or three presutural intralars; three postsutural dorsocentrals, two strong and two small presutural dorsocentrals; three to four presutural acrostichals, two or three postsutural acrostichals of which one or two are strong, including the prescutellar bristles; two marginal scutellars, a small apical pair and a strong preapical pair of bristles. Propleura and prosternum bare.

Abdomen gray with changeable stripes and a median black stripe on the second and third tergites; series of marginal bristles on the fourth and fifth tergites. Sternites with long black pile and small bristles on the margin of the sternites two to four, fifth sternites strongly excised. *Forcipes superiores* curved forward with small spines on the back; *f. inferiores* almost triangular; penis with bilobed spinous ventralia.

Legs black, middle femur with two bristles on the anterior side; two preapicals on the posterior side; an apical ctenidium of four bristles on the ventral side. Hind femur with two series of bristles, the inferior one composed of small bristles; two series of bristles on the ventral side.. Middle tibia with a long median bristle on the anterior side; one bristle below the middle on the ventral side. Hind tibia with one bristle on the ventral side. Wings hyaline, veins yellow, costal spine very small; R_{4+5} with bristles on the half or two-thirds way to crossvein. Segments of costal vein as follows: II : 37, III : 16, IV : 55, V : 20, VI : 3.

Holotype and paratype two males from Wollogorang, N. Australia, 9.IX.1930, T.G. Campbell. The holotype was returned to Camberra, the paratype was placed in the collections of the Instituto Oswaldo Cruz (nr. 8.412).

This species was named in honor of G. H. Hardy.

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